Changes in response to insulin and the effects of varying glucose tolerance on whole-body protein metabolism in patients with cystic fibrosis

Mahroukh Rafii, Karen Chapman, Cynthia Stewart, Erin Kelly, Amir Hanna, David C Wilson, Elizabeth Tullis, and Paul B Pencharz

ABSTRACT
Background: Diabetes mellitus has been reported to increase whole-body protein breakdown and thus loss of lean body mass. Cystic fibrosis–related diabetes (CFRD) is associated with undernutrition and increased mortality.

Objective: We hypothesized that CFRD is associated with increased whole-body protein breakdown, which results in negative protein balance, and that correction of the glucose intolerance with insulin therapy would normalize whole-body protein metabolism.

Design: Rates of whole-body protein turnover and protein balance were measured in 28 adults with cystic fibrosis (17 M, 11 F). Subjects were assessed with a modified oral-glucose-tolerance test and categorized as having normal glucose tolerance, impaired glucose tolerance, or CFRD with and without fasting hyperglycemia; then they were compared with previously diagnosed CFRD adults already receiving insulin therapy. Indexes of protein turnover were calculated from [15N]glycine and [15N]urea.

Results: Analysis of variance for the 28 subjects showed that whole-body protein breakdown was highest (P < 0.05) in patients with CFRD. Whole-body protein synthesis was not significantly affected by impaired glucose tolerance. Significant (P < 0.05) improvement in net protein synthesis occurred in the CFRD group 3 mo after insulin therapy was administered. Follow-up studies of 3 subjects with CFRD showed significant improvement in net protein synthesis after insulin therapy. Monitoring of the protein homeostasis of the impaired glucose tolerance group gave clues to the progression of their metabolic homeostasis.


KEY WORDS Cystic fibrosis, cystic fibrosis–related diabetes, glucose tolerance, whole-body protein metabolism, protein breakdown, protein synthesis

INTRODUCTION
As more people with cystic fibrosis (CF) are living into adulthood, CF–related diabetes (CFRD) is becoming much more common, affecting up to 25% of adults with CF (1). In CF, the problem is usually one of energy balance—that is, the protein intake is adequate, but energy imbalance occurs as a result of the combination of poor energy intake, increased energy losses, and increased energy needs (2). Retrospective studies of adults with CFRD show that, for several years before the clinical onset of CFRD, patients lose weight and their lung disease worsens. However, after insulin treatment, both their nutritional and clinical status reverts toward normal (4). Diabetes, in general is known to have adverse effects on protein balance (5). Similarly, we have shown that energy imbalance has an adverse effect on protein metabolism (6, 7). We conducted a retrospective survey in which CFRD patients were shown to have 25% mortality over a 5-y period, whereas the CF patients without diabetes had 11% mortality in the same time span, after correction for age and pancreatic status (3).

The effects of CFRD on protein and energy metabolism are poorly understood at this time. Because, in retrospective studies, clinical deterioration of patients precedes the diagnosis of CFRD, we reason that metabolic disturbances occur early in the evolution of CFRD, before the manifestations of disease (3). Our group has developed techniques for assessing protein (8) and energy (7, 9) metabolism in a noninvasive way, and thus our goal in the current study was to ascertain the effects of preclinical intermittent hyperglycemia and of newly diagnosed CFRD on the energy and protein metabolism and body composition of persons with CF. On the basis of our earlier studies in idiopathic diabetes, we expected to find whole-body protein breakdown (WBPB) to be increased but synthesis to be unchanged, which would result in a negative protein balance.

In earlier work that was conducted in patients with adult-onset noninsulin–dependent diabetes, we (10) were able to separate glucose homeostasis from protein balance. We showed that exogenous insulin was needed to normalize protein metabolism, whereas energy restriction alone was sufficient to normalize glucose metabolism. Thus a corollary objective of the current...
study was to ascertain the effects of insulin treatment on the protein and energy metabolism and body composition both in patients with CFRD and in those with intermittent hyperglycemia.

SUBJECTS AND METHODS

Subjects

Thirty-three CF patients were enrolled from the adult CF clinic at St. Michael’s Hospital, Toronto. Pancreatic-insufficient adults with CF, whose glucose tolerance had recently been assessed by a modified oral-glucose-tolerance test (OGTT), were recruited between January 1998 and November 2002. All adults with CF have their glucose tolerance screened on an annual basis unless they have developed diabetes and are taking insulin. The pancreatic phenotype—ie, pancreatic-sufficient or pancreatic-insufficient—of all CF patients at our clinic was established (11). Clinical data are kept in the Toronto CF Database (Mary Corey, coordinator), which has collected prospective data since 1977. Glucose tolerance was tested when the patients were clinically stable (ie, >1 mo after the last acute pulmonary exacerbation). Patients were excluded if they were pregnant or were receiving corticosteroid therapy. On the basis of the results of their annual modified OGTT, patients were categorized into 4 groups (12–14) in order of decreasing glucose tolerance: 1) normal glucose tolerance (NGT) group, which had fasting glucose concentrations < 7.0 mmol/L and 2-h plasma glucose concentrations <7.8 mmol/L; 2) impaired glucose tolerance (IGT) group, which had fasting glucose concentrations <7.0 mmol/L and 2-h glucose concentrations between 7.8 and 11.1 mmol/L; 3) diabetic glucose tolerance without fasting hyperglycemia (CFRD–no FH) group, which had fasting glucose concentrations <7.0 mmol/L and 2-h glucose concentrations >11.1 mmol/L; and 4) diabetic glucose tolerance with fasting hyperglycemia (CFRD-FH) group, which had fasting glucose concentrations >7.0 mmol/L and 2-h glucose concentrations >11.1 mmol/L.

An initial study of patients in each group was conducted. A fifth group of adults with CF, previously diagnosed with CFRD and taking insulin (CFRD-I group), was also studied for comparison purpose. Follow-up studies at 3 mo and 6 mo after the initial study were performed on 5 IGT, 2 CFRD–no FH, and 2 CFRD-FH subjects. As is standard practice in our center, the 2 subjects with CFRD–no FH were started on home glucose monitoring; over time, this monitoring found fasting hyperglycemia, and thus, by the time of the 3 mo follow-up study, these subjects, along with the 2 CFRD-FH subjects, were receiving insulin therapy. Diabetes management was carried out by an endocrinologist (AH), using standard insulin therapy and home glucose monitoring (14). A total of 52 studies were carried out. Five of the 33 initial studies were not used in the analysis because of noncompliance (ie, missing urine samples or food records). Four of 19 follow-up studies were excluded for similar reasons. The study was performed while the subjects were outpatients at the Clinical Investigation Unit at The Hospital for Sick Children, Toronto.

Each subject was told the purpose of the study and the possible risks involved, and written informed consent was obtained. The subjects received financial compensation for their food and travel costs. All procedures were approved by the Research Ethics Committee at St. Michael’s Hospital.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject characteristics at initial study†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>Age</td>
</tr>
<tr>
<td>NGT (6 M, 2 F)</td>
<td>27.1 ± 9.3</td>
</tr>
<tr>
<td>IGT (4 M, 4 F)</td>
<td>32.8 ± 10.8</td>
</tr>
<tr>
<td>CFRD (1 M, 3 F)</td>
<td>41.0 ± 7.4</td>
</tr>
<tr>
<td>CFRD-I (6 M, 2 F)</td>
<td>32.1 ± 9.2</td>
</tr>
</tbody>
</table>

† All values are ± SD. NGT, normal glucose tolerance; IGT, impaired glucose tolerance; CFRD, cystic fibrosis–related diabetes (with and without hyperglycemia); CFRD-I, previously diagnosed CFRD taking insulin therapy. Means in the same column with different superscript letters are significantly different, P < 0.05. (one-factor ANOVA and Tukey’s studentized range post hoc test).

Experimental design

The study was conducted in 2 parts. The first part of the study aimed to ascertain whether glucose intolerance has a significantly more adverse effect on protein and energy metabolism in patients with CFRD or intermittent hyperglycemia, or both, than it has on subjects in a normoglycemic PI disease control (NGT) group. Subjects were placed in the appropriate glucose tolerance category before the day of the study. Studies were carried out during a 1-d visit to the Clinical Investigation Unit at the Hospital for Sick Children. The second part of the study included all subjects who had IGT, namely, those diagnosed with CFRD and IGT. The CFRD groups were started on insulin and restudied at 3 and 6 mo. The IGT subjects were monitored for glucose tolerance and restudied at 3 and 6 mo.

Subjects arrived at ~0800, having fasted for the previous 12 h. A baseline urine sample was collected at the first void. Weight was measured to the nearest 0.1 kg with a balance-beam scale (Detecto; Cardinal Scales, Webb City, MO). Standing height was measured to the nearest 0.1 cm with a wall-mounted stadiometer (Holtain Ltd, Crymmych, United Kingdom). The characteristics of 28 subjects at these initial studies are shown in Table 1.

Midupper arm circumference (MAC) and skinfold thicknesses (triceps, biceps, subscapular, and suprailliac) were measured to the nearest 1 mm with Harpenden calipers to obtain estimates of fat mass and, by subtracting fat mass from body weight, of fat-free mass (15). Total body water was measured by bioelectrical impedance analysis (16). Resistance (R) and reactance (Xc) measurements were made by using a 4-terminal bioelectrical impedance analyzer (model 101A; RJL Systems, Detroit), and equations were used to predict lean body mass (16–18). The body-composition data from the initial studies are shown in Table 2.

Resting energy expenditure (REE) was then measured by using computerized open-circuit indirect calorimetry (Sensormedics 2900; Sensormedics, Anaheim, CA) while the patient was under a ventilated hood for a minimum of 30 min. The system used a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer. Air flow was adjusted with a mass flow sensor. Results were expressed in kilojoules per day and also as a percentage of the predicted REE values for age, weight, and sex as derived from the FAO/WHO/UNU predictive equations (19). The energy values are shown in Table 3.

Whole-body protein metabolism (WBPM) component was ascertained by administering a single dose of [15N]glycine and measuring the cumulative excretion of [15N]urea in urine. The method was described by Waterlow et al (20) and modified by...
TABLE 2

Body composition of subjects at initial study

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Fat-free body mass</th>
<th>Fat mass</th>
<th>Percentage fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT (n = 8)</td>
<td>50.6 ± 5.3</td>
<td>12.6 ± 5.0</td>
<td>19.6 ± 6.2</td>
</tr>
<tr>
<td>IGT (n = 8)</td>
<td>43.8 ± 14.8</td>
<td>12.3 ± 3.4</td>
<td>20.3 ± 5.6</td>
</tr>
<tr>
<td>CFRD (n = 4)</td>
<td>46.6 ± 3.7</td>
<td>10.1 ± 0.7</td>
<td>17.9 ± 2.1</td>
</tr>
<tr>
<td>CFRD-I (n = 8)</td>
<td>57.0 ± 8.0</td>
<td>17.4 ± 6.8</td>
<td>22.8 ± 6.6</td>
</tr>
</tbody>
</table>

1 All values are ± SD. NGT, normal glucose tolerance; IGT, impaired glucose tolerance; CFRD, cystic fibrosis-related diabetes (with and without hyperglycemia); CFRD-I, previously diagnosed CFRD taking insulin. None of the differences were significant (one-factor ANOVA and Tukey’s studentized range post hoc test).

TABLE 4

Whole-body protein metabolism at initial study

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Dietary protein intake</th>
<th>Whole-body protein synthesis</th>
<th>Whole-body protein breakdown</th>
<th>Net synthesis (g protein · kg⁻¹ · d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT (n = 8)</td>
<td>1.97 ± 0.71</td>
<td>3.55 ± 1.32</td>
<td>2.56 ± 1.23</td>
<td>1.00 ± 0.70</td>
</tr>
<tr>
<td>IGT (n = 8)</td>
<td>2.16 ± 0.79</td>
<td>2.63 ± 1.05</td>
<td>1.87 ± 0.65</td>
<td>0.87 ± 0.70</td>
</tr>
<tr>
<td>CFRD (n = 4)</td>
<td>1.64 ± 0.13</td>
<td>4.41 ± 0.84</td>
<td>3.98 ± 0.92</td>
<td>0.43 ± 0.12</td>
</tr>
<tr>
<td>CFRD-I (n = 8)</td>
<td>1.79 ± 0.24</td>
<td>3.46 ± 0.98</td>
<td>2.61 ± 1.07</td>
<td>0.84 ± 0.37</td>
</tr>
</tbody>
</table>

1 All values are ± SD. NGT, normal glucose tolerance; IGT, impaired glucose tolerance; CFRD, cystic fibrosis-related diabetes (with and without hyperglycemia); CFRD-I, previously diagnosed CFRD taking insulin. Means in the same column with different superscript letters are significantly different, P < 0.05 (one-way ANOVA and Tukey’s studentized range post hoc test).

The effect of insulin treatment on follow-up studies

The effect of insulin on the body composition and the protein and energy metabolism in the newly diagnosed CFRD-FH patients and the CFRD-no FH patients.

RESULTS

A total of 28 subjects were included in analyses. Five subjects were excluded from the analyses because of incomplete food records or incomplete urine collections.

Subject characteristics and body composition

Subject characteristics at initial study are shown in Table 1. Clearly, the most difficult subjects to recruit in the 4-y study period were those in the CFRD–no FH and CFRD-FH subgroups of the CFRD category. Because their numbers were low and because the 2 subjects with CFRD–no FH (established by home glucose monitoring) progressed to CFRD-FH and thus needed insulin, we decided to pool the 2 groups into one CFRD group. We therefore had 3 groups with increasing glucose intolerance; the fourth group was the previously diagnosed CFRD patients who were already taking insulin therapy, the CFRD-I group. There was no significant difference in age between the 4 groups. Body weight tended to be lower as glucose tolerance status worsened. In contrast, no difference in body composition was noted between the 4 groups (Table 2).

Energy and protein metabolism

No significant differences were seen in energy intake. Although the REE and the percentage of the predicted REE values in the CFRD-I group tended to be higher than those values in the other groups, the differences were not significant (Table 3). WBPM data are shown in Table 4. WBPP was highest in the CFRD group but significantly (P < 0.05) different only from that in the IGT group. The CFRD-I group did not differ significantly from the NGT group. Synthesis was numerically greater in the new CFRD-FH patients and the CFRD–no FH patients.

Effect of insulin treatment on follow-up studies

The effect of insulin on the body composition and the protein and energy metabolism in the CFRD group at the 3- and 6-mo
follow-up is shown in Table 5. Three of 4 subjects in this group were included in the follow-up analyses. Results for the 4th subject were omitted from the final analyses because of her deteriorating condition, which was due to lung failure (she died soon after her 6-mo visit). There tended to be an improvement in WBPB but no significant change in synthesis 3 mo after the administration of insulin. Net synthesis improved significantly at 3 mo after the beginning of insulin therapy, mainly because of an improvement in protein breakdown (Table 5). Although body weight, BMI, and fat-free body mass measurements improve numerically on treatment, the changes did not reach significance on repeated-measures ANOVA in the short follow-up time of 6 mo. However, fat mass and percentage fat improved significantly after 6 mo of therapy. There was no effect of insulin treatment on REE or percentage of predicted REE values.

Follow-up studies in the impaired group

Changes in protein breakdown in the IGT group at the 3- and 6-mo follow-up are shown in Figure 1. Two of 5 patients showed worsening of their breakdown, which most likely represented a progression toward moderate glucose impairment—ie, they moved into the CFRD–no FH category. Yet 2 other patients showed an improvement in their proteolysis, and the OGTT reclassified the 5th subject to the NGT group. Body composition, energy, and other protein data did not change significantly at follow-up and are not displayed.

DISCUSSION

The purpose of the first part of the study was to examine whether glucose intolerance has an adverse effect on body composition, protein, and energy metabolism in subjects with CF. Our longer-term goal was to determine whether treatment of hyperglycemia in CFRD patients will correct abnormal WBPM (if present) and improve nutritional status.

Understanding the nature of CFRD is critical for patients with CF, because the additional diagnosis of diabetes is associated with greater nutritional failure, worse pulmonary disease, and earlier death (1, 3, 24). Retrospective studies showed that pulmonary decline and weight loss begin 2–4 y before the diagnosis of CFRD (1, 4), and mortality is dramatically higher in CF patients with diabetes than in the general CF population. CFRD is a distinct form of diabetes seen in pancreatic-insufficient CF patients. CFRD is categorized differently from type 1 or 2 diabetes, although it shares features of both (25); its primary cause is insulin deficiency, although there is also a degree of insulin resistance (14, 26–28).

Our data show that there was no significant effect of glucose intolerance on the body-composition and energy variables. However, abnormalities in glucose balance had an adverse effect on protein metabolism. Furthermore, the results in the CFRD group showed a positive effect of insulin therapy on fat mass and protein metabolism (ie, an improvement in net synthesis) over the 3- and 6-mo follow-up. The CFRD consensus conference recommended that adults with CF undergo an annual modified OGTT and that those with CFRD–no FH be carefully followed with home glucose monitoring, whereas those with CFRD-FH should be started on insulin therapy (14). Clearly, the CFRD–no FH group is of particular interest. In an unpublished survey at our clinic, that group constituted 9.9% of all patients, and, over a year of follow-up, one-third of those 9.9% experienced worsening glucose tolerance or death. In the 2 subjects with CFRD–no FH whom we recruited on the basis of their annual modified OGTT results, their glucose tolerance worsened such that, by the time of their follow-up studies, they were taking insulin. It is important to note that, at the time of their initial study, they were undergoing glucose monitoring and were still categorized as CFRD–no FH. A numerical increase in WBPB was evident in all 4 subjects with CFRD whether they had fasting hyperglycemia or not.

Because glucose tolerance worsened in this small sample of patients, we were unable to show any decrease in BMI or lean body mass; yet, when the glucose-impaired (CFRD) group was compared with the CFRD-I group, the latter group showed a significantly greater increase in weight than did the former group. These findings are in agreement with those of earlier studies of protein metabolism in diabetic patients (29). Although body weight, BMI, and lean body mass measurements tended to
improve progressively with insulin treatment, they did not differ significantly at the end of the 6-mo follow-up. Further, our results did show significant improvement in fat mass and percentage fat after only 6 mo of insulin therapy. Perhaps a longer follow-up period and a larger sample size would have shown a greater effect of insulin on the body-composition variables, an effect comparable to that reported earlier (4). Langn et al (24) found that, after 2 y of insulin therapy in CFRD patients, BMI and lung function had decreased to the same values as in the non-diabetic control CF patients. CFRD has been associated with weight loss and general clinical decline, especially in the months leading up to the diagnosis (24). Langn et al evaluated the effect of evolving CFRD on clinical and nutritional status. Thirty-eight patients with CFRD were retrospectively compared with 38 matched non-diabetic NGT CF patients. Significant differences in body weight and BMI emerged 4 y before the diagnosis of CFRD. In a follow-up study by the same investigators, 18 patients who had received insulin therapy for CFRD for at least 2 y were matched with 18 non-diabetic CF control patients (4). After 2 y of insulin, BMI had improved in the CFRD group such that it was similar to BMI in the CF controls.

Diabetes is known to have adverse effects on protein balance (5)—that is, on the difference between whole body protein synthesis and breakdown. In healthy adults, protein breakdown and protein synthesis are in equilibrium to maintain protein balance. Several factors that can disturb normal protein balance have been postulated to be relevant in patients with CF, including insulin deficiency, malnutrition, infection, and the basic CF cellular defect. In the last 30 y, 2 major methods have been developed to measure rates of protein flux, synthesis, and degradation by using amino acids labeled with stable isotopes of carbon and nitrogen. To measure WBPM in patients with CF, we have used a modified end-product method because it is noninvasive, avoids the need for hospitalization, and has the ability to assess results during the integrated feeding-fasting that represents the nonsteady condition of real life (21). The only disadvantage of this method is that a 48-h urine collection is time consuming and requires subject compliance. Other groups have used the precursor method to estimate WBPM (30–32). In that method, the kinetics of an indispensable amino acid, usually leucine, is studied, and the results are extrapolated to WBPM. That method is invasive and requires hospital admission. In addition, the subjects are studied only in the postabsorptive state, which does not represent daily life (8, 21).

On the basis of evidence from our earlier studies of type 2 diabetes, we expected to find in this current study that WBPM increased but synthesis was relatively unchanged, which would result in a negative protein balance (10, 33). Our data on WBPM showed that the 4 subjects with newly diagnosed CFRD had the highest breakdown rates. However, these values differed significantly only from those in the IGT group, and they may reflect the small sample size. Conversely, when these subjects were treated with insulin, their protein metabolism normalized and net protein synthesis increased significantly as a result of a decrease in breakdown rates (Table 5). These findings are also in agreement with those from studies performed by Hardin et al (34). Two other research groups have asked similar questions by using invasive techniques such as the hyperinsulinenemic-euglycemic clamp (28, 29). However, their studies of protein metabolism were restricted to the fasting state (28), whereas our method provides a 24-h integration of fasting and fed periods (8) and hence presents a complete picture of daily nutrient balance. Because the results from the 2 methods lead to similar conclusions, the fasting studies appear to provide data that are applicable to the whole 24-h period. A study by Moran et al (27) extended earlier observations by others (30, 31) and showed that the inability to spare protein in the fasted state (fasted with exogenous insulin) is due to a combination of defective insulin secretion and resistance to the suppressive action of insulin on protein catabolism. It appears that the reduced action of insulin may contribute to net protein proteolysis. However, when sufficient exogenous insulin is given, as evidenced by data from our CFRD-I group, it is clear that breakdown is normalized and is similar to that in the glucose control NGT group. We also observed the same reduction in WBBP in patients newly diagnosed with CFRD who were treated with insulin. This finding is in agreement with our earlier work, conducted in patients with type 2 diabetes, which showed that glucose homeostasis can be corrected by using high-protein, very-low-energy diets (33). However, it was not until insulin was given to the subjects that the protein balance was restored to normal through suppression of the higher WBPP (10, 29). Our findings agree with the reasoning that ensuring the optimal well-being of patients with CFRD involves ensuring that their protein metabolism is fully corrected, which appears to require exogenous insulin.