Protein Metabolism in Clinically Stable Adult Cystic Fibrosis Patients With Abnormal Glucose Tolerance

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Cystic fibrosis (CF) patients are reported to experience chronic protein catabolism. Since diabetes or impaired glucose tolerance (IGT) is common in CF, we hypothesized that their protein catabolic state is related to reduced insulin secretion or reduced insulin action. A total of 12 clinically stable adult CF patients with abnormal glucose tolerance and 12 age-, sex-, and lean body mass–matched healthy control subjects underwent protein turnover studies using L-[1-^{13}C]leucine, L-[^{15}N] phenylalanine, and L-[$^{2}H_4$]tyrosine, with and without exogenous insulin infusion. In the baseline fasting state, protein metabolism was entirely normal in CF patients, with no evidence of increased protein catabolism. In contrast, striking abnormalities were seen in CF patients when insulin was infused, since they did not experience normal suppression of the appearance rates of leucine, phenylalanine, or tyrosine (indexes of protein breakdown). At an insulin concentration of 45 ± 2 μU/ml, normal control subjects suppressed the leucine appearance rate by 19 ± 5% ($P < 0.01$), ketoisocaproate appearance rate by 10 ± 3% ($P = 0.03$), tyrosine appearance rate by 11 ± 2% ($P = 0.03$), and phenylalanine appearance rate by 6 ± 3% ($P = 0.07$). Phenylalanine conversion to tyrosine decreased by 22 ± 7% ($P = 0.03$). At a similar insulin concentration of 44 ± 3 μU/ml, normal suppression of amino acid appearance did not occur in CF. The leucine appearance rate decreased by 4 ± 2% ($P = 0.65$), ketoisocaproate appearance rate by 1 ± 2% ($P = 0.94$), tyrosine appearance rate by 0 ± 6% ($P = 0.56$), phenylalanine appearance rate by 5 ± 6% ($P = 0.34$), and phenylalanine conversion to tyrosine by 5 ± 6% ($P = 0.95$). Poor suppression of the amino acid appearance rate in CF was not related to previously documented glucose tolerance status (IGT or CF-related diabetes without fasting hyperglycemia), fasting insulin levels, the acute insulin response, insulin sensitivity, cytokine or counterregulatory hormone levels, resting energy expenditure, caloric intake, pulmonary function, or clinical status. Protein synthesis was not significantly affected by insulin infusion in either normal control subjects or CF patients. In conclusion, clinically stable adult CF patients have normal indexes of protein breakdown and synthesis in the fasting state. In contrast, elevation of plasma insulin to physiological postprandial levels fails to normally suppress indexes of protein breakdown. It is therefore likely that inability to spare protein during the postprandial state is the cause of protein catabolism in these patients. *Diabetes* 50:1336–1343, 2001

Impairment of insulin secretion is nearly universal in adult cystic fibrosis (CF) patients with pancreatic exocrine insufficiency (1–5). A spectrum of glucose tolerance abnormalities is seen, depending on the degree of islet destruction, the insulin sensitivity of the individual (5–10), and possibly genetic factors associated with islet amyloid deposition (11). Approximately 15% of adult CF patients have CF-related diabetes (CFRD) with fasting hyperglycemia (FH) (fasting plasma glucose ≥ 126 mg/dl or 7.0 mmol/l) and require insulin either chronically or intermittently to control fasting glucose levels (12). About one quarter of adult CF patients have CFRD without fasting hyperglycemia (12). They have normal fasting glucose levels, but their 2-h glucose levels are >200 mg/dl (11.1 mmol/l) during standard oral glucose tolerance tests (OGTTs). Impaired glucose tolerance (IGT), defined as a 2-h OGTT glucose level between 140 and 200 mg/dl (7.8–11.1 mmol/l), occurs in about one-third of adult CF patients (12). CF patients with IGT or CFRD without FH are not usually treated with insulin because they have reasonably normal glucose levels when measured by meter at home, normal HbA_{1c} levels, and absence of significant glycosuria (12,13). It is not known, however, whether the insulin-deficient state in CF has deleterious effects unrelated to glucose metabolism.

With improvements in medical care over the last 30 years, the average life expectancy of patients with CF is now 31 years, with many living into their fourth and fifth decades (14). For reasons that are not well understood, the diagnosis of diabetes is associated with significantly increased mortality in CF (14–17). The Cystic Fibrosis Foundation, which maintains a database on >21,000 North American patients, reports a sixfold greater mortality in CF patients with diabetes (14). Prospective evaluation of patients who differed at baseline only in the degree of glucose tolerance abnormalities showed that the rate of decline in pulmonary function over a 4-year observation period was lowest in subjects with normal glucose tolerance at baseline, significantly greater in those with IGT, and greatest in those with CFRD with FH (15). The rate of

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AIR, acute insulin response; CF, cystic fibrosis; CFRD, CF–related diabetes; CRC, clinical research center; DEXA, dual-energy X-ray absorptiometry; FEV_1, forced expiratory volume in 1 s; FFM, fat-free mass; FH, fasting hyperglycemia; GCMS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; IGFBP3, IGF-binding protein 3; IGT, impaired glucose tolerance; NIH, National Institutes of Health; OGTT, oral glucose tolerance test; REE, resting energy expenditure; S_G, glucose effectiveness; S_I, insulin sensitivity; TNF, tumor necrosis factor.

pulmonary decline was inversely related to the magnitude of insulin secretion at baseline, suggesting a cause-and-effect relationship between insulin deficiency and CF clinical deterioration.

How might insulin deficiency adversely affect clinical status in CF? Insulin is a potent anabolic hormone that plays a pivotal role in carbohydrate, protein, and lipid metabolism, and severe insulin deficiency is associated with significant protein catabolism (18). Negative protein balance may contribute to morbidity and mortality in CF, since lung function correlates with lean body mass (19), and reversal of protein catabolism stabilizes pulmonary function and decreases the number of hospitalizations for acute pulmonary exacerbation (20). We hypothesized that because of insufficient insulin secretion, a protein catabolic state is present in clinically stable adult CF patients with abnormal glucose tolerance. To test this hypothesis, we used stable isotopes of amino acids to comprehensively assess CF protein turnover.

RESEARCH DESIGN AND METHODS

Subjects. The 450 patients followed at the University of Minnesota CF Center routinely undergo annual OGTTs. CF patients who had been diagnosed in the previous 6 months with either IGT ($n = 6$) or CFRD without FH ($n = 6$) were recruited into the present study. There is potential for variability in OGTT classification, even when the test is repeated within days, but the OGTT was used to identify insulin-deficient patients who did not have FH. Patients were excluded who had a history of fasting hyperglycemia, supplemental oxygen dependency, CF liver disease, or who had experienced acute illness (as defined in the national multicenter DNase study [21]), oral or intravenous glucocorticoid therapy, or a weight change >5% during the preceding 3 months. Because the CF study subjects were chronically infected with CF pathogens, they all received chronic suppressive aerosolized antibiotic preparations. Healthy nonathlete normal control subjects were recruited through poster advertisement. Approval for these studies was obtained from the University of Minnesota Committee for the Use of Human Subjects in Research, and informed consent was obtained from all subjects.

General study design. Subjects were studied at the University of Minnesota General Clinical Research Center (CRC) over a 3-day period. They did not eat or drink each night from 9:00 P.M. until 10:00 A.M., but otherwise had unlimited access to food. Dietary intake during the admission was recorded by the CRC dietary staff and was compared with baseline nutrient and protein intake as assessed by food records collected for 3 days before the study. During the week before admission, subjects did not undergo any unusual exertion.

Two indwelling catheters were inserted in each subject, one in an arm vein for delivery of study infusates and the other in a contralateral dorsal hand vein for blood sampling. The blood-sampling catheter was inserted retrograde and the hand placed in a heating pad during the metabolic studies. Protein turnover was studied on the first day in the fasting state and was repeated on the third day during insulin infusion. On the second day, insulin secretion and insulin sensitivity (S_I) were measured. Patients were given their usual morning medications and bronchial drainage therapies each day after the morning studies were completed.

Protein metabolic studies

Protocol for isotope administration on days 1 and 3. Stable isotopes of leucine, phenylalanine, and tyrosine were used to measure indexes of protein catabolism (amino acid rate of appearance), protein synthesis, and amino acid oxidation. The theoretical basis of the use of these isotopes is reviewed elsewhere (22). All isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA) and were prepared into solutions under sterile conditions. At time 0 (midnight), after baseline samples were obtained, subjects received the following priming doses: L-[1-^{13}C]leucine, 1.0 mg/kg; [^{13}C]sodium bicarbonate, 0.2 mg/kg; L-[^{15}N]phenylalanine, 0.6 mg/kg; L-[^{15}N]tyrosine, 0.2 mg/kg; and L-[^{2}H$_4$]tyrosine, 0.5 mg/kg. Isotope was infused at a constant rate using a volumetric infusion pump for 10 h. The isotope infusion consisted of [1-^{13}C]leucine, 1.0 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}; L-[^{15}N]phenylalanine, 0.6 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}; and L-[^{2}H$_4$]tyrosine, 0.5 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}. Plasma studies for isotopic enrichment of [^{13}C]leucine, [^{13}C]ketoisocaproate, [^{15}N]phenylalanine, [^{15}N]tyrosine, and [^{2}H$_4$]tyrosine, and breath samples for analysis of $^{13}CO_2$ enrichment were obtained at 15-min intervals from 8:00 to 10:00 A.M.

On day 2, at 6 P.M., a 16-h insulin infusion was begun at 0.05 U $\cdot$ kg^{-1} $\cdot$ h^{-1}. Dextrose was infused at a rate adjusted to maintain the serum glucose level at 100 mg/dl. Isotope infusion and sample collection were repeated on day 3 during the insulin infusion.

Measurement of plasma isotopic enrichment. The isotopic enrichment of [^{13}C]leucine was determined using an HP5988 gas chromatography–mass spectrometry (GCMS) by multiple ion monitoring under positive ion methane chemical ionization conditions (23). L-[^{15}N]phenylalanine, L-[^{15}N]tyrosine, and L-[^{2}H$_4$]tyrosine were measured as their *t*-butyl ether derivatives under electron ionization conditions (24). [1-^{13}C]-α-ketoisocaproate in plasma was determined as its quinoxalinol-tri-methyl-silyl derivative under electron ionization conditions, using an HP MS Engine GCMS. L-[^{2}H$_4$]tyrosine was measured after derivatization with tertiary butyldimethylsilyl ether under electron ionization conditions at M/Z at 466/467/470, [M-57]fragment ions. Expired air samples were collected in a special bag and transferred to a 20-ml vacutainer tube. [^{13}C]bicarbonate enrichment in blood and breath samples was measured with an isotope ratio mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany) (25). Plasma concentrations of amino acids were determined by high-performance liquid chromatography (HPLC) (26).

Calculation of protein turnover using isotopic enrichments. Amino acid flux for leucine, phenylalanine, and tyrosine was calculated as follows:

$$Q = i(E_i/E_p - 1)$$

where Q = flux, i = infusion rate of tracer, E_i = enrichment of infusate, and E_p = plasma enrichment at plateau. Calculations were made for leucine kinetics using both leucine and ketoisocaproate. Nonoxidative disposal of leucine is used as an indicator of protein synthesis, since it is assumed that leucine has no fates other than irreversible oxidative loss and incorporation into protein. Using this assumption, protein synthesis equals leucine flux minus leucine oxidation. Leucine oxidation was calculated from the following formula:

$$Ox_{leu} = F \times (1/E_p - 1/E_i) \times 100$$

where Ox_{leu} = leucine oxidation, F = [^{13}C]bicarbonate production above baseline (V_{CO_2} × breath enrichment [^{13}C]bicarbonate/weight), and E_p is the plasma enrichment of ketoisocaproate at steady state (22,27).

Phenylalanine flux (Q_p) and tyrosine flux (Q_t) were calculated by isotope dilution using the formula described above. Phenylalanine in the free amino acid pool has only two fates: It is either converted to tyrosine or incorporated into body protein (a measure of protein synthesis). Thus, protein synthesis from phenylalanine = Q_p–Q_{pt},where Q_{pt} = conversion of phenylalanine to tyrosine (28);

$$Q_{pt} = Q_t \times (E_t/E_p) \times Q_p/(i_p + Q_p)$$

where E_t = plasma enrichment tyrosine, E_p = plasma enrichment phenylalanine, and i_p = rate of infusion of phenylalanine. The expression $Q_p/(i_p + Q_p)$ corrects for the contribution of the infusion to Q_{pt}. The details of this equation are discussed elsewhere (28).

The terminology "protein breakdown" (whole body) may be used in the discussion portion of this manuscript in lieu of leucine or phenylalanine appearance rate, because the appearance rate of these amino acids is an index of protein breakdown. This assumption is based on our knowledge that in the fasted state, leucine appears in the free amino acid pool only from protein breakdown, since leucine is not synthesized in vivo in humans. Similarly, phenylalanine, another essential amino acid, also is not synthesized in the body, and its appearance rate represents protein breakdown. The nonoxidative leucine flux and the portion of phenylalanine flux that is not converted to tyrosine is considered as protein synthesis. The details of the assumptions involved in these terminologies are discussed elsewhere (22,27,28).

Nutrition and illness covariate measurements. Insulin, glucagon, growth hormone, IGF-I, and IGF-binding protein 3 (IGFBP3) were measured by standard radioimmunoassays. IGF-I and IGFBP3 were measured by the Nichols Institute (San Juan Capistrano, CA). Epinephrine and norepinephrine were measured by thin-layer chromatography (29). Tumor necrosis factor (TNF)-α, interleukin-6, and interferon-γ were measured in the Cytokine Reference Laboratory (University of Minnesota) by enzyme-linked immunosorbent assay using commercial kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Normal reference ranges were established by the Cytokine Reference Laboratory. HbA_{1c} was measured by HPLC, C-reactive protein by rate nephelometry, and cortisol by fluorescence polarization immunoassay.

Computerized indirect calorimetry (Delta Trac II; Sensor Medics) was used as a standard measure of resting energy expenditure (REE) and cellular oxygen consumption (V_{O_2}). Dual-energy X-ray absorptiometry (DEXA) was used to assess body composition using a Lunar Radiation (Madison, WI) DPX DEXA system. The patient's perception of his or her state of health was assessed using the SF-36 Health Survey, which was developed and validated

TABLE 1
Subjects

	Age (Years)	Sex (F/M)	Weight (kg)	Height (cm)	BMI (kg/m^2)	FFM (kg)	% FFM
Control subjects	28 ± 1	6/6	56.6 ± 1.7	165 ± 2	20.7 ± 0.3	44.4 ± 3.1	78 ± 3
CF patients	29 ± 2	6/6	56.4 ± 1.8	164 ± 2	21.0 ± 0.5	46.7 ± 2.6	83 ± 3

Data are n or means ± SD.

during the Medical Outcomes Study (30) but has not been validated in CF. A higher SF-36 score indicates a better-perceived health state.

Clinical status of CF patients was assessed with the modified National Institutes of Health (NIH) score (31,32). This score assigns points to various measures of chronic and acute health, including pulmonary function, chest X-ray changes, physical examination, sputum production, pulmonary events such as hemoptysis or pneumothorax, weight loss, appetite, dyspnea, fatigue, and others. The highest possible score of 100 indicates no health problems. A score of 91–100 suggests excellent health; 81–90, very good health; 71–80, good health; 61–70, fair health; and an NIH score ≤60 suggests poor health with a high likelihood of death within the next 3 years (32).

Insulin secretion and sensitivity. On day 2 of hospitalization, the acute insulin response (AIR) was measured, and S_I and glucose effectiveness (S_G) were estimated using an exogenous insulin administration-modified, frequently sampled intravenous glucose tolerance test (33). At time 0, subjects received glucose (20 g intravenous bolus dose). Glucose and insulin levels were measured at −10, −5, 0, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 19 min. At time 20 min, insulin (0.03 U/kg) was given as an intravenous bolus dose. Glucose and insulin levels were measured at 22, 24, 26, 28, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 min. Bergman's minimal model of glucose kinetics was used to estimate study parameters (33,34). C-peptide levels were not measured, but previous studies have shown excellent correlation between C-peptide and insulin levels in CF (1). Because we did not measure C-peptide levels in the present study, however, we cannot exclude the possibility that differences in suppression of endogenous insulin secretion could have resulted in differences in portal insulin concentration, which might have influenced protein turnover.

Statistical analysis. The data on the variables measured are presented as means ± SE. Data that did not demonstrate a normal distribution are presented as medians (range). Comparisons between CF patients and control subjects for baseline characteristics and the different protein catabolism indexes were performed by Student's t test or by the nonparametric median test. Paired t tests were used to investigate changes in amino acid appearance rate in response to insulin infusion within each group. In addition, differences in the amino acid appearance rate from baseline in response to insulin infusion were compared between the study groups by t test. One-way analysis of variance was used to test for differences in mean AIR, S_I, and S_G between control subjects and the two glucose tolerance categories of CF patients. Linear regression was used to investigate for possible correlation among AIR, S_I, S_G, and the indexes of protein catabolism. This was also done to investigate for possible relationships between amino acid rates of appearance and the nutritional and illness covariates measured. The nonparametric Spearman's rank correlation was where skewness was detected in the data. Differences between the groups in the changes in amino acid levels after insulin infusion were analyzed by rank analysis of covariance, adjusting for baseline levels as a covariate. For all analyses performed, a P value of 0.05 was used as the cutoff for statistical significance.

RESULTS

Subjects. CF patients and normal control subjects were matched for age, sex, weight, height, BMI, and fat-free mass (FFM) (Table 1). No CF patient had signs or symptoms of acute illness in the 3 months preceding the study. All of them were engaged in relatively normal lifestyles and were either college students or employed full-time. Of the cytokines and counterregulatory hormones measured, only α-interferon was significantly elevated in CF (Table 2), supporting our clinical observation that there was no evidence of acute or severe infection. Predictably, the SF-36 Health Survey showed that CF patients had more concerns about their state of health than did normal control subjects. A range in pulmonary function was seen, with the forced expiratory volume in 1 s (FEV_1) varying from 41 to 104% predicted. Despite poor pulmonary func-

TABLE 2
Markers of infectious status and overall health

	Control subjects	CF patients
TNF (pg/ml)	3.0 (0–7.2)	0.9 (0–15.3)
Interleukin-6 (pg/ml)	1.3 (0–7.7)	3.2 (0.9–14.5)
α-Interferon (pg/ml)	0.02 ± 0.02	2.63 ± 0.81*
C-reactive protein (mg/dl)	0.3 (0–0.9)	0.2 (0–1.8)
Epinephrine (pg/ml)	61 (25–260)	64 (42–358)
Norepinephrine (pg/ml)	173 (99–536)	232 (80–411)
Cortisol (μg/dl)	15 ± 1	18 ± 1
Glucagon (pg/ml)	56 ± 7	54 ± 13
Growth hormone (μg/l)	4 ± 1	3 ± 1
Fasting insulin (μU/ml)	5 ± 2	4 ± 1
Fasting glucose (mg/dl)	90 ± 8	101 ± 6
IGF-I (ng/ml)	205 ± 13	206 ± 14
IGFBP3 (mg/l)	2.28 ± 0.24	2.52 ± 0.15
REE	1271 ± 56	1546 ± 65*
REE/kg FFM	31 ± 1	33 ± 1
V_{O_2}	184 ± 8	220 ± 9*
V_{CO_2}	161 ± 7	196 ± 8*
RQ	0.87 ± 0.01	0.90 ± 0.01
SF-36 score	89 ± 1	69 ± 5†
NIH score	NA	76 ± 1
FEV_1 % predicted	NA	68 ± 7

Data that were not normally distributed are medians (range); otherwise, data are means ± SE. *$P < 0.01$ and †$P < 0.001$ CF patients vs. control subjects.

TABLE 3
Insulin secretion and sensitivity by minimal model analysis

	S_i	S_g	AIR (mU/ml)	HbA_{1c} (%)
Control subjects	4.3 ± 0.4	221 ± 15	680 ± 78	5.0 ± 0.1
CF patients	3.6 ± 0.5	180 ± 17	187 ± 34*	5.7 ± 0.1*

Data are means ± SE. *$P < 0.001$ CF patients vs. control subjects.

tion in many patients, none had a poor NIH score. No differences existed between the CF glucose tolerance groups in nutritional status, markers of infectious status, or overall health.

Energy expenditure. When assessed by 3-day dietary records performed at home, both total caloric intake and protein intake were higher in CF patients compared with matched control subjects (control: 2,019 ± 177; CF: 3,005 ± 308 kcal/day, ~48% difference, $P < 0.01$). CF patients are frequently asked to record dietary intake as part of their routine clinical care, and all of the CF subjects had previous experience with this procedure. During the CRC visit, where dietary intake could be more accurately quantitated, the difference in caloric and protein intake between CF patients and control subjects largely disappeared. This finding suggests possible underreporting by normal control subjects or overreporting by CF patients at home or that control subjects ate more than usual in the CRC setting (control: 2,600 ± 198; CF: 3,098 ± 288 kcal/day, $P = 0.14$). The CRC caloric intake of normal control subjects was ~35% higher than that reported at home, whereas there was little difference between the CRC caloric intake and that reported at home by the CF patients. The proportion of calories derived from protein, fat, and carbohydrate was similar between the two groups (protein 16–18%, fat 31–35%, and carbohydrate 49–58% of total caloric intake). We did not measure stool fat content, but it can be assumed that some portion of the dietary intake was not absorbed in CF patients, since 5–15% of fat is not absorbed despite the use of exogenous pancreatic enzyme supplementation (35,36). None of our patients had obvious symptoms of malabsorption (abdominal pain or malodorous, greasy, or floating stools). Although the absolute REE was higher in CF patients, it was normal when expressed per FFM (Table 2). Oxygen consumption (V_{O_2}) and carbon dioxide production (V_{CO_2}) were both ~20% higher in CF patients than in control subjects (Table 2). No difference was seen in respiratory quotient.

Insulin secretion and sensitivity. Fasting insulin levels were normal in CF (Table 2). As expected, AIR was significantly lower in CF patients than in control subjects (Table 3). S_I and S_G were normal in CF patients. There was no correlation between AIR, S_I, or S_G and any of the cytokine or counterregulatory hormone levels, REE, calorie intake, FEV_1, or clinical status (SF-36 Health Survey or NIH scores). HbA_{1c} was significantly elevated in CF, although still within the laboratory's normal range (<6.0%).

Baseline fasting protein metabolism. In the postabsorptive (fasting) state, protein metabolism was entirely normal in CF patients (Table 4). Leucine kinetics were evaluated using both the enrichment of leucine and that of ketoisocaproate. Ketoisocaproate is a metabolite formed intracellularly by the transamination of leucine. Since protein turnover occurs in the intracellular space, isotopic enrichment of ketoisocaproate may more accurately reflect total-body protein breakdown than enrichment of plasma leucine (24,37). Protein breakdown (as reflected by leucine, tyrosine, and phenylalanine rates of appearance), leucine oxidation, and protein synthesis did not differ between CF patients and normal control subjects.

TABLE 4
Postabsorptive (fasting) protein turnover in CF (μmol · kg $FFM^{-1} \cdot h^{-1}$)

	Control subjects	CF patients
Indexes of protein breakdown		
Amino acid appearance rate (μmol · kg $FFM^{-1} \cdot h^{-1}$)		
[^{13}C]KIC	126 ± 7	128 ± 4
[^{13}C]Leucine	107 ± 9	99 ± 4
[^{2}H4]Tyrosine	50 ± 3	52 ± 4
[^{15}N]Phenylalanine	56 ± 3	51 ± 3
Indexes of protein synthesis		
Nonoxidative leucine flux (μmol · kg $FFM^{-1} \cdot h^{-1}$)		
[^{13}C]KIC	101 ± 6	98 ± 5
[^{13}C]Leucine	83 ± 6	75 ± 4
Phenylalanine disappearance not accounted for by conversion to tyrosine (μmol · kg $FFM^{-1} \cdot h^{-1}$)		
[^{15}N]Phenylalanine	50 ± 3	44 ± 3
Indexes of amino acid catabolism		
Leucine oxidation (μmol · kg $FFM^{-1} \cdot h^{-1}$)		
[^{13}C]KIC	28 ± 2	30 ± 3
Phenylalanine conversion to tyrosine (μmol · kg $FFM^{-1} \cdot h^{-1}$)	7 ± 1	6 ± 1

Data are means ± SE.

Fasting protein metabolism in CF patients was not related to previously demonstrated glucose tolerance status (IGT versus CFRD without FH), fasting insulin levels, AIR, S_I, S_G, or any of the cytokine or counterregulatory hormone levels, REE, calorie intake, FEV_1, or clinical status (SF-36 Health Survey or NIH scores).

Protein metabolism in response to insulin infusion. Baseline insulin concentrations on day 1 averaged 4 ± 1 μU/ml (24 ± 6 pmol/l) in CF patients and 5 ± 2 μU/ml (30 ± 12 pmol/l) in control subjects. Insulin concentrations from 8:00 to 10:00 A.M. on day 3, after an overnight insulin infusion, averaged 44 ± 3 μU/ml (264 ± 18 pmol/l) in CF patients and 45 ± 2 μU/ml (270 ± 12 pmol/l) in control subjects.

As expected, normal control subjects suppressed amino acid appearance rates in response to insulin (Table 5). Control leucine rate of appearance calculated from leucine enrichment dropped from 107 ± 9 to 88 ± 5 μmol · kg $FFM^{-1} \cdot h^{-1}$ ($P < 0.01$). Control leucine rate of appearance calculated from ketoisocaproate enrichment dropped from 126 ± 7 to 110 ± 6 μmol · kg $FFM^{-1} \cdot h^{-1}$ ($P < 0.03$). Tyrosine rate of appearance dropped from 50 ± 3 to 43 ± 2 μmol · kg $FFM^{-1} \cdot h^{-1}$ ($P = 0.03$). Phenylalanine rate of appearance also suppressed in response to insulin infusion in control subjects, but the difference did not achieve

TABLE 5
Protein turnover during insulin infusion (plasma insulin level 44 ± 3 μU/ml in CF patients and 45 ± 2 μU/ml in control subjects)

	Control subjects	CF patients
Indexes of protein breakdown		
Amino acid flux (μmol · kg FFM^{-1} · h^{-1})		
[^{13}C]KIC	110 ± 6*	128 ± 5†
[^{13}C]Leucine	88 ± 5*	95 ± 5
[^{2}H4]Tyrosine	43 ± 2*	51 ± 4†
[^{15}N]Phenylalanine	50 ± 2	52 ± 4
Index of protein synthesis		
Phenylalanine disappearance not accounted for by conversion to tyrosine (μmol · kg FFM^{-1} · h^{-1})‡		
[^{15}N]Phenylalanine	46 ± 3	44 ± 4
Index of amino acid catabolism		
Phenylalanine conversion to tyrosine (μmol · kg FFM^{-1} · h^{-1})	5 ± 0*	6 ± 1†

Data are means ± SE. *$P < 0.05$ compared with day 1; †$P < 0.05$ control subjects vs. CF patients; ‡Synthesis and oxidation were not calculated from the leucine or KIC data because glucose was infused during the insulin infusion, which results in contamination from exogenous [^{13}C]leucine.

statistical significance (phenylalanine: 56 ± 3 to 50 ± 2 μmol · kg FFM^{-1} · h^{-1}, $P = 0.07$).

In contrast to the normal protein breakdown indexes seen in the baseline fasting state, striking abnormalities were seen in CF patients when insulin was infused. CF patients did not show the expected suppression of amino acid appearance in response to insulin (Fig. 1). CF leucine rate of appearance calculated from leucine enrichment went from 99 ± 4 to 95 ± 5 μmol · kg FFM^{-1} · h^{-1} ($P = 0.65$). CF leucine rate of appearance calculated from ketoisocaproate enrichment went from 128 ± 4 to 128 ± 5 μmol · kg FFM^{-1} · h^{-1} ($P = 0.94$). Similarly, no difference was seen in tyrosine or phenylalanine rate of appearance.

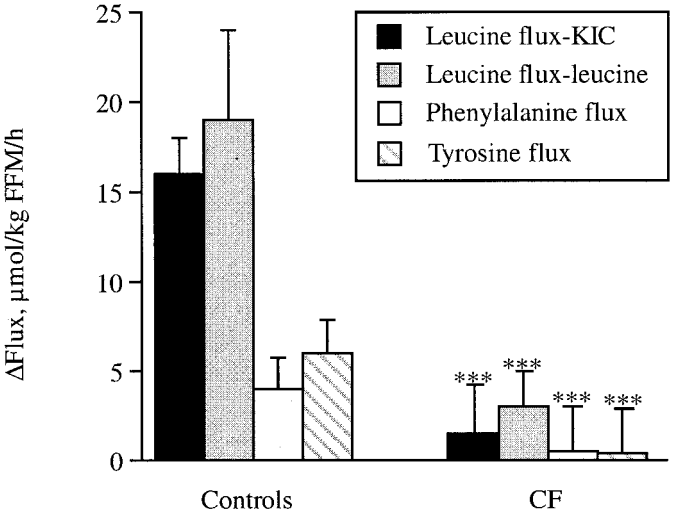

FIG. 1. The magnitude of suppression of protein breakdown (amino acid flux) after insulin infusion, with steady-state insulin levels of 44 ± 3 μU/ml from a baseline of 4 ± 1 μU/ml (CF patients) and 45 ± 2 μU/ml from a baseline of 5 ± 2 μU/ml (control subjects). ***$P < 0.001$ compared with control subjects.

TABLE 6
Amino acid levels at baseline (day 1) and during insulin infusion (day 3)

	Day 1		Day 3	
	CF patients	Control subjects	CF patients	Control subjects
Asparagine	165 ± 7	177 ± 20	116 ± 17*	155 ± 24
Glutamate	175 ± 12	175 ± 12	145 ± 18*	143 ± 11
Serine	152 ± 24	135 ± 19	68 ± 19*	74 ± 10*
Glutamine	626 ± 47	548 ± 66	428 ± 68*	472 ± 60*†
Histidine	69 ± 7	77 ± 9	69 ± 13	50 ± 6*
Glycine	348 ± 22	392 ± 43	263 ± 29*	308 ± 20*
Threonine	197 ± 18	228 ± 27	120 ± 15*	135 ± 12*
Alanine	450 ± 69	400 ± 62	314 ± 54*	316 ± 21
Arginine	294 ± 17	289 ± 29	197 ± 12*	239 ± 22
Tyrosine	70 ± 9	54 ± 8	45 ± 13	34 ± 16*
Valine	210 ± 33	186 ± 25	83 ± 17*	97 ± 16*
Methionine	22 ± 6	28 ± 5	26 ± 12	22 ± 6
Phenylalanine	65 ± 9	56 ± 5	35 ± 8*	37 ± 3*
Isoleucine	41 ± 4	42 ± 8	21 ± 2*	23 ± 5*
Leucine	110 ± 4	155 ± 13†	55 ± 11*	67 ± 6*
Lysine	126 ± 10	170 ± 12†	69 ± 9*	78 ± 11*

Data are means ± SE. *$P < 0.05$ suppression from baseline values; †$P < 0.05$ control subjects vs. CF patients.

Phenylalanine conversion to tyrosine dropped from 6.5 ± 0.5 to 4.7 ± 0.5 μmol · kg FFM^{-1} · h^{-1} ($P = 0.03$) in control subjects, while no change was seen in CF (6.4 ± 0.5 to 6.3 ± 0.7 μmol · kg FFM^{-1} · h^{-1}). Protein synthesis, calculated from phenylalanine flux minus phenylalanine conversion to tyrosine, was not significantly affected by insulin infusion in either normal control subjects or CF patients (Table 5).

Poor suppression of protein breakdown in CF patients was not related to previously demonstrated glucose tolerance status (IGT versus CFRD without FH), fasting insulin levels, AIR, S_I, S_G, any of the cytokine or counterregulatory hormone levels, REE, caloric intake, pulmonary function (FEV_1), or clinical status (SF-36 Health Survey or NIH scores).

Amino acid levels. At baseline in the fasting state, lysine and leucine levels were lower in CF than in control subjects (CF lysine 126 ± 10, control lysine 161 ± 15, $P = 0.04$; CF leucine 119 ± 8, control leucine 146 ± 14, $P = 0.05$) (Table 6). All other baseline amino acid levels were comparable. After insulin infusion, there were no significant differences in amino acid levels between CF patients and control subjects. In response to insulin, plasma levels of all amino acids declined in CF except asparagine, methionine, histidine, and tyrosine. In controls subjects, levels of all amino acids expect asparagine, methionine, glutamic acid, alanine, and arginine declined. After adjusting for baseline insulin levels, CF subjects had significantly greater insulin-induced suppression of glutamine levels than did control subjects ($P = 0.02$). Suppression of all other amino acids was similar.

DISCUSSION

In the current study, a comprehensive assessment of protein metabolism was performed in patients with CF, using several different isotopes of amino acids to measure protein turnover. The nutritional, infectious, and glucose metabolic status of these patients was well characterized.

In the fasting state, protein metabolism was entirely normal in clinically stable, well-nourished CF adults with abnormal glucose tolerance. In contrast, even after prolonged insulin infusion, CF patients did not experience normal suppression of protein breakdown (as reflected by lack of suppression of leucine, tyrosine, and phenylalanine appearance rates). Normally, insulin levels are elevated after food intake, which leads to insulin-induced suppression of endogenous protein breakdown, resulting in net protein anabolism (38). The current study demonstrates that patients with CF do not appropriately suppress protein breakdown in response to insulin, and thus, this mechanism of preserving protein mass is defective in people with CF.

A previous study of adult CF patients used the leucine rate of appearance to demonstrate increased protein breakdown in the fasting state (39). In contrast, the current study, using the rates of appearance of three isotopes as indicators, found fasting protein breakdown to be completely normal in CF. The differences between the two study populations may help to clarify the etiology of protein catabolism in some patients with CF. Although our subjects had significant lung disease (with FEV_1 values as low as 41% predicted), none had an NIH score <65 or a cytokine pattern suggestive of severe infection. Insulin sensitivity was normal. The subjects in the previous study (39) had elevated TNF levels (Dr. Dana Hardin, personal communication), insulin resistance (10), and, in ~20% of patients, NIH scores <65. Both insulin resistance and fasting leucine rate of appearance correlated with NIH clinical status score. Poor NIH scores, insulin resistance, and elevated TNF levels all suggest a greater degree of underlying infection in their subjects, and point to severe inflammation as the primary cause of fasting protein catabolism in some CF patients.

Despite resistance to insulin-induced suppression of proteolysis, CF patients in the present study were sensitive to insulin stimulation of peripheral glucose disposal. Similarly, we have shown by the euglycemic clamp technique that clinically stable adult CF patients with normal insulin-induced stimulation of peripheral glucose disposal have hepatic insulin resistance, with poor suppression of hepatic glucose production in response to insulin (6). This discordant insulin action in CF suggests that there may be a hierarchy in the metabolic systems affected by factors causing insulin resistance, and that when insulin resistance factors are subtle, peripheral glucose metabolism in response to insulin remains intact. Thus, isolated postprandial defects in insulin suppression of proteolysis and increased hepatic glucose production may be signs of mild insulin resistance in CF related to chronic low-grade inflammation and infection. The insulin-resistant state may progress to fasting protein catabolism and peripheral insulin resistance as patients become more ill.

It was reported that supraphysiological levels of insulin (100 μU/ml) are able to normalize suppression of amino acid appearance rates in CF patients (39). The insulin level achieved in the current study (45 ± 2 μU/ml) is consistent with normal physiological elevations in healthy subjects after a mixed meal. The lack of suppression of amino acid appearance rates at these insulin levels indicates that people with CF may not experience the normal postprandial anticatabolic effect of insulin. This problem may be compounded in CF by the fact that postprandial insulin levels are usually diminished due to poor insulin secretion. Thus, new protein accretion in CF may be hampered by both poor insulin secretion and diminished ability of insulin to suppress endogenous protein breakdown. This assumption is supported by findings of impaired suppression of proteolysis after a mixed meal in young children with CF (40).

In healthy adults, protein metabolism is a complex process in which protein breakdown and protein synthesis are in equilibrium to maintain protein balance. A variety of nutritional, hormonal, and pathological factors may have an impact on this system. Several factors that can disturb normal protein balance have been postulated to be relevant in the patient with CF, including insulin deficiency, malnutrition, infection, and the basic CF cellular defect. It is intriguing that the baseline postabsorptive protein turnover was normal in people with CF in the current study. It is possible that FFM as measured by DEXA scan may have overestimated lean tissue in CF patients if they had increased water retention, as is observed in some populations such as the elderly (22). If this were the case, we may have underestimated fasting protein catabolism in CF. The current study measured only indexes of whole-body protein turnover, which is an average turnover of different body proteins. Only studies involving measurement of different protein fractions could demonstrate whether in CF patients the abnormalities during insulin infusion occurred to all proteins or only to some fractions of proteins. If the different protein fractions have differential responses to insulin and their turnover rates are different in CF patients and control subjects, the whole-body measurements may underestimate protein turnover, and this underestimation may increase with prolonged duration of isotope infusion.

In the presence of absolute insulin deficiency (type 1 diabetes), increased protein breakdown and body protein loss occur (41). Lean body mass and protein turnover are normal in individuals with type 2 diabetes (42), where insulin deficiency is only relative because of insulin resistance. Thus, it may be that excessive net protein catabolism is only apparent in the complete absence of insulin. Our data in CF patients support these observations. Although they have diminished insulin secretion in response to oral and intravenous stimuli, basal fasting insulin levels are generally normal in people with CF, as noted in our subjects, even in the presence of diabetes (1). In the fasting state in healthy CF adults, there appears to be enough insulin present to suppress excessive protein catabolism, despite IGT or diabetes without FH. However, unlike patients with type 2 diabetes (43) and healthy control subjects (38,43), CF patients failed to normally inhibit protein breakdown in response to insulin.

Previous studies of protein metabolism in CF have yielded conflicting information, and have often been difficult to interpret due to differences in the ages, nutritional status, and infectious status of the patients as well as the use of different measurement techniques (20,39,40,44–49). The majority of previous studies of protein balance in CF were performed on growing children. Unlike adults, in whom protein catabolism and synthesis are balanced,

healthy children are in positive nitrogen balance, where synthesis outweighs catabolism because of growth and development. Thus, information about protein turnover gathered from children with CF may not be relevant in CF adults. While normal rates of protein breakdown are described for well-nourished CF children (20,44,45), there are several reports of increased protein catabolism in malnourished growth-retarded children with CF (20,44, 46,47). This latter finding may be more related to infection than to poor nutritional status, since malnourished children identified as being free of infection had normal protein breakdown (45).

Plasma concentrations of several amino acids were lower in patients with CF. When the effect of insulin on plasma phenylalanine concentration was assessed, there was a 46% decrease with insulin in control subjects, but only a 33% decrease observed in CF patients. This observation is consistent with the fact that less suppression of phenylalanine appearance occurred in CF patients. In the case of tyrosine and leucine, the concentrations of these amino acids depend on metabolic processes that were not measured in this study (tyrosine catabolism, leucine oxidation, and leucine transamination). Of note, the decline in glutamine concentration in response to insulin was greater in CF patients than in control subjects (31 vs. 16%, respectively). The precise mechanism for the greater decline in CF patients remains to be defined, but continued consumption of glutamine for gluconeogenesis in CF patients is a likely explanation. Reduced insulin-induced suppression of hepatic glucose production has been previously shown in CF patients (6), which supports the likelihood of continued gluconeogenesis in CF patients during insulin infusion as is seen in type 2 diabetic patients (50).

In conclusion, in the absence of significant underlying infection, clinically stable adult CF patients have normal amino acid appearance rates in the fasting state, even when severe abnormalities in insulin secretion and significant impairment of pulmonary function are present. In contrast, normal suppression of amino acid appearance (reflecting inhibition of protein breakdown) does not occur when insulin levels are elevated within the physiological postprandial range. This result indicates that in the fed state, the combination of defective insulin secretion plus resistance to the suppressive action of insulin on protein breakdown may contribute to net protein catabolism in these patients. Administration of insulin during meals may lead to greater suppression of endogenous protein breakdown and favor net protein synthesis in the fed state.

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