Insulin Regulation of Free Fatty Acid Kinetics in Adult Cystic Fibrosis Patients With Impaired Glucose Tolerance

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Cystic fibrosis (CF) patients are insulin-resistant with regards to suppression of hepatic glucose production and proteolysis, but the effect of insulin on adipose free fatty acid (FFA) release has not been studied. [9,10-^{3}H]palmitate kinetics were measured in 11 stable adult CF patients with impaired glucose tolerance (IGT) and 9 normal control subjects. Baseline plasma palmitate concentrations [CF = 99 ± 13 (median 74, range 65 to 187); control = 88 ± 9 (88, 46 to 138) μmol/L, *P* = .9] and palmitate flux [CF = 114 ± 11 (100, 72 to 171); control = 105 ± 12 (106, 54 to 182) μmol/min, *P* = 0.9] were not different between CF patients and controls. During a euglycemic clamp with infusion of insulin to physiologic postprandial levels, however, palmitate concentrations tended to be higher in CF patients: CF = 18 ± 3 (13, 10 to 47), control = 12 ± 1 (11, 8 to 18) μmol/L, *P* = 0.08. The higher palmitate concentrations during hyperinsulinemia appeared to be due to reduced suppression of adipose tissue palmitate release, because mean palmitate flux was 33% greater in CF subjects [32 ± 5 (26, 17 to 66) μmol/min] than controls: [24 ± 2 (23, 17 to 34) μmol/min], *P* = .20. There was considerably greater heterogeneity in insulin-induced suppression of plasma palmitate concentration and flux in CF patients compared to normal control subjects. In summary, a defect in insulin suppression of lipolysis was seen in clinically stable CF patients with IGT, similar to what has been described in CF for amino acid and glucose metabolism. This quantitative difference in lipolysis may account for inadequate insulin-induced suppression of hepatic glucose production in CF, and may be a metabolic adaptation to increased energy needs.

CYSTIC FIBROSIS (CF) is the most common lethal autosomal recessive disease of Caucasians, affecting 1 in 3,000 individuals. Defects in the CF transmembrane regulator alter chloride, sodium, and water movement across epithelial cell membranes and produce viscous, dehydrated secretions. Poor mucociliary clearance in the lungs leads to chronic pulmonary infection and inflammation. Many other organ systems are affected, including the exocrine and endocrine pancreas, hepatobiliary tract, gut, and reproductive organs. Undernutrition is common, and is due to increased energy needs, malabsorption, and appetite disturbances. While most of these patients die of end-stage pulmonary disease, survival is intimately related to nutritional status.[1] Thus, an understanding of CF fuel metabolism is critical.

Multiple defects in substrate utilization have been described in CF. Of these, abnormal glucose tolerance is the most apparent. Only about 25% of adult CF patients have normal glucose tolerance, while 35% have impaired glucose tolerance (IGT), 25% have diabetes without fasting hyperglycemia, and 15% have diabetes with fasting hyperglycemia.[2] First-phase insulin secretion is absent in most individuals with CF, and the insulin response to oral stimuli is delayed and blunted.[3] The primary defect is insulin deficiency, caused at least in part by fibrotic damage to the pancreas.[4] Variable insulin resistance is also present. Peripheral glucose disposal has been reported to be normal, increased, or decreased, depending on the degree of accompanying acute and chronic inflammation.[2,5-10] In contrast, even patients with normal peripheral insulin sensitivity are insulin-resistant with respect to suppression of hepatic glucose production (HGP).[2]

Recent studies have demonstrated that CF patients with abnormal glucose tolerance experience accelerated protein catabolism.[11,12] The physiology of CF could, therefore, mimic the physiology of other insulin-resistant conditions. CF patients are sometimes malnourished, and, thus, semistarvation might be one model with potential similarities to CF. However, while prolonged fasting in humans leads to accelerated lipolysis and insulin resistance with regards to glucose and free fatty acid (FFA) metabolism, there is relative protection of body protein stores.[13] Insulin-resistant conditions that are associated with increased proteolysis, such as obesity,[14-16] severe inflammation,[17-20] and type 2 diabetes,[21-23] are generally associated with increased lipolysis, increased HGP, and diminished peripheral glucose disposal. Whether insulin-deficient CF patients have abnormalities of lipolysis similar to those observed with other pathologic conditions has not been studied. Defining this pattern in CF could help develop testable hypotheses regarding the mechanism for the insulin resistance with respect to glucose metabolism. To address this issue, isotope dilution studies of FFA kinetics were undertaken in a group of CF patients with IGT and a matched control group.

MATERIALS AND METHODS

Subjects

The 450 patients followed at the University of Minnesota CF Center routinely undergo annual oral glucose tolerance testing (OGTT) after the age of 6 years. CF patients who had been diagnosed in the previous 6 months with IGT (fasting plasma glucose <126 mg/dL, 2-hour post-load glucose 140 to 200 or 1-hour glucose >200 mg/dL) were

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recruited from clinic. Patients were excluded who had a history of fasting hyperglycemia, supplemental oxygen dependency, or CF liver disease, or who had received oral or intravenous glucocorticoid therapy or had a weight change of greater than 5% during the preceding 3 months. All CF patients are chronically infected with typical CF pathogens such as *Pseudomonas*, and all at the University of Minnesota receive chronic suppressive aerosolized antibiotic preparations. No patient, however, had experienced an acute pulmonary exacerbation or a change in their routine antibiotic regimen for at least 3 months before the study. Healthy, non-athlete normal control subjects were recruited by poster advertisement. Approval for these studies was obtained from the University of Minnesota Committee for the Use of Human Subjects in Research; informed consent was obtained from all subjects.

Study Design

Subjects were admitted in the evening to the University of Minnesota General Clinical Research Center. Two indwelling catheters were inserted in each subject, one in an arm vein for delivery of study infusate 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. Subjects were given nothing by mouth after 9 PM.

[9,10-^{3}H]palmitate (New England Nuclear, Boston, MA) was used to measure FFA turnover.[13,24] The isotope was prepared into solution under sterile conditions using 5% albumin. After baseline laboratory studies were obtained, [9,10-^{3}H]palmitate was infused at a constant rate of 0.3 μCi/min using a volumetric infusion pump for 6 hours (5 AM to 11 AM). The postabsorptive (fasting) state was studied from time 0 to 3 hours. A euglycemic, hyperinsulinemic clamp was started at 3 hours. Insulin, 0.5 mU/kg/min, was infused from time 3 hours to 6 hours to evaluate insulin suppression of palmitate turnover. This dose of insulin was chosen to obtain plasma insulin concentrations typical of those found postprandially,[24] because postprandial suppression of lipolysis is an important contributor to changes in metabolic function.

Plasma glucose concentrations were measured at 5-minute intervals during the insulin infusion, and an infusion of 20% dextrose was constantly adjusted to maintain the serum glucose concentration at 100 mg/dL (5.6 mmol/L). Plasma samples were obtained at baseline and at 15-minute intervals from 2 hours to 3 hours (steady-state postabsorptive period) and from 5 hours to 6 hours (steady-state hyperinsulinemia), for measurement of palmitate concentrations and specific activity (SA), as well as insulin concentrations. Samples were collected on ice, centrifuged at 4°C, and stored at −70°C.

Plasma palmitate SA and concentrations were determined using high-performance liquid chromatography (HPLC). Chemical and isotopic purity were tested in these isotopes prior to use and all solutions were prepared under sterile conditions and were free of bacteria and pyrogens before use. The [9,10-^{3}H]-palmitate was bound to albumin in preparation for infusion. Plasma palmitate concentration and SA were measured by HPLC using [$^2H_{31}$] palmitate as an internal standard as previously described.[25-27] The coefficient of variation for replicate analysis using this procedure is 3.8% for concentration and 2.3% for SA. Steady-state palmitate flux is calculated as the rate of ^{3}H palmitate infusion (dpm/min) ÷ mean plasma ^{3}H palmitate SA (dpm/μmol). Thus, palmitate turnover is presented as μmol/min, which, if the study groups have similar resting energy expenditure, is the optimal means of data expression.[28]

Peripheral glucose metabolism was evaluated by determining the exogenous glucose infusion (M, mg/kg/min) required to maintain euglycemia during the last 40 minutes of the clamp.

Insulin,[29] glucagons,[30] growth hormone,[31] and insulin-like growth factor-I (IGF-I)[32] concentrations were measured by standard radioimmunoassay and cortisol by fluorescence polarization immunoassay.[33] Computerized indirect calorimetry (DeltaTrac II, SensorMedics, Yorba Linda, CA) was used to measure resting energy expenditure (REE).[34] Dual energy x-ray absorptiometry (DEXA) was employed to assess body composition using a Lunar Radiation Corp (Madison, WI) instrument.

Statistical Analysis

The data on the variables measured are presented as means ± SEM and also, when significant skew was present, as medians (range). Comparisons between characteristics at baseline were performed by Student's *t* test. Due to skewness in the distributions of palmitate concentration and flux data for the CF patients, comparisons with the control group were done by nonparametric tests. Differences at baseline were analyzed by the Mann-Whitey test. Differences between the study groups in the change in palmitate metabolism from baseline metabolism compared to the response to insulin infusion were analyzed by rank analysis of covariance, adjusting for baseline levels of palmitate concentration and palmitate flux as covariates. For all the analyses performed, a *P* value of .05 was used as the cutoff for statistical significance.

RESULTS

Subjects

Eleven CF patients were compared to nine normal control subjects of similar age, gender, race, weight, height, body mass index (BMI), and fat-free mass (FFM) (Table 1). Cortisol, glucagons, and growth hormone levels were normal in CF patients, supporting the clinical observation that there was no evidence of acute or severe infection. Fasting insulin and IGF-I levels were also normal in CF, as is typical for the University of Minnesota CF population. Cholesterol levels were normal to low in the CF patients (median = 126 [range = 117 to 175] mg/dL), while triglyceride levels were elevated to greater than 200 mg/dL in 3 of the 8 patients in whom they were available (135 [84 to 291] mg/dL).

OGTT

Fasting glucose levels ranged from 89 to 108 mg/dL in CF patients (Table 1). The area under the 2-hour OGTT curve for glucose was 9,413 ± 734 mg/dL and the area under the curve for insulin was 2,992 ± 866 μU/mL. Normal control subjects did not undergo OGTT testing, but in a previous study at the University of Minnesota,[3] normal control subjects of similar age (24 ± 1 years) and BMI (21 ± 1 kg/m^2) had about one third the glucose excursion (3,221 ± 851 mg/dL) and substantially greater insulin excursion (4,569 ± 598 μU/mL) of CF patients in the current study. Thus, as has been previously reported,[3] abnormal glucose tolerance in the subjects with CF was related to deficient insulin secretion.

Peripheral Glucose Metabolism

M, the amount of glucose required to maintain euglycemia during the final 40 minutes of the insulin infusion, was lower in CF: controls 8.1 ± 2.3, CF 5.4 ± 1.4 mg/kg/min ($P = .01$). Thus, peripheral insulin sensitivity to glucose metabolism was decreased in the CF subjects. Dividing M by the steady-state insulin concentration blunted this conclusion: controls 0.26 ± 0.05, CF 0.21 ± 2.3 mg/kg/min/μU/mL ($P = .8$).

Table 1. Baseline Subject Characteristics

	CF (n = 11)	Controls (n = 9)
Age (yr)	25 ± 7	24 ± 6
Gender	5F, 6M	4F, 5M
Weight (kg)	62 ± 9	62 ± 9
Height (cm)	169 ± 8	170 ± 11
BMI (kg/m^2)	21.7 ± 1.7	21.2 ± 1.3
Fat-free mass (kg)	48 ± 9	47 ± 12
Cortisol (μg/dL)	8 ± 6	12 ± 4
Glucagon (pg/mL)	101 ± 28	84 ± 15
Growth hormone (μg/L)	2 ± 3	2 ± 1
Fasting glucose (mg/dL)	98 ± 6	94 ± 6
Fasting insulin (μU/mL)	7 ± 3	10 ± 3
Cholesterol (mg/dL)*	126 (117-175)	—
Triglycerides (mg/dL)*	135 (84-291)	—
IGF-I (ng/mL)	217 ± 98	220 ± 68
Fasting respiratory quotient	.89 ± .04	0.85 ± .05
Fasting REE (kcal)	1,543 ± 178	1,453 ± 324

NOTE. Data are presented as mean ± SD, or, when significant skew was present, as median (range).
*Available in 8 patients

Plasma Palmitate Concentrations and Flux in the Baseline Postabsorptive State

Baseline plasma palmitate concentrations after an overnight fast were not significantly different between CF patients and control subjects: CF = 99 ± 13 (median 74, range 65 to 187), control = 88 ± 9 (88, 46 to 138) μmol/L, $P = .9$ (Fig 1A). Baseline palmitate flux, reflecting lipolysis, was also similar between the 2 groups: CF = 114 ± 11 (100, 72 to 171), control = 105 ± 12 (106, 54 to 182) μmol/min, $P = .9$ (Fig 1B).

We recently reported that REE is the best way to examine overnight postabsorptive FFA flux;[28] therefore, palmitate flux was plotted versus REE for the participants in this study (Fig 2). Consistent with our previous observations, there was a positive relationship between REE and palmitate flux ($r = 0.39$, $P = .08$).

Plasma Palmitate Concentrations and Palmitate Flux in Response to Insulin Infusion

During the euglycemic, hyperinsulinemic clamp, plasma insulin concentrations rose from baseline values of 7 ± 3 μU/mL to 31 ± 3 μU/mL in CF patients and from 10 ± 3 μU/mL to 32 ± 3 μU/mL in control subjects ($P = .8$). Palmitate concentrations were significantly suppressed compared to baseline in both CF patients and control subjects in response to insulin ($P < .001$) (Fig 3A). However, less suppression occurred in the CF patients, since their plasma palmitate concentrations were about 50% higher than those of control subjects during insulin infusion: CF = 18 ± 3 (median 13, range 10 to 47), control = 12 ± 1 (11, 8 to 18) μmol/L, $P = .08$. The greater palmitate concentration in CF patients was due to impaired suppression of adipose tissue lipolysis rather than to decreased clearance.

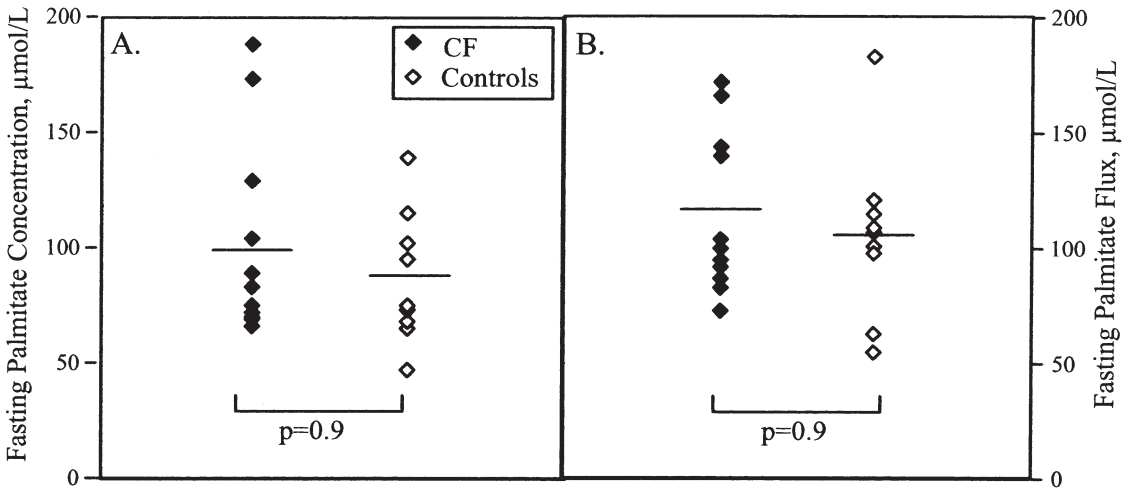

Fig 1. Baseline fasting palmitate concentration (A) and flux (B) in CF patients and control subjects. The line indicates the mean.

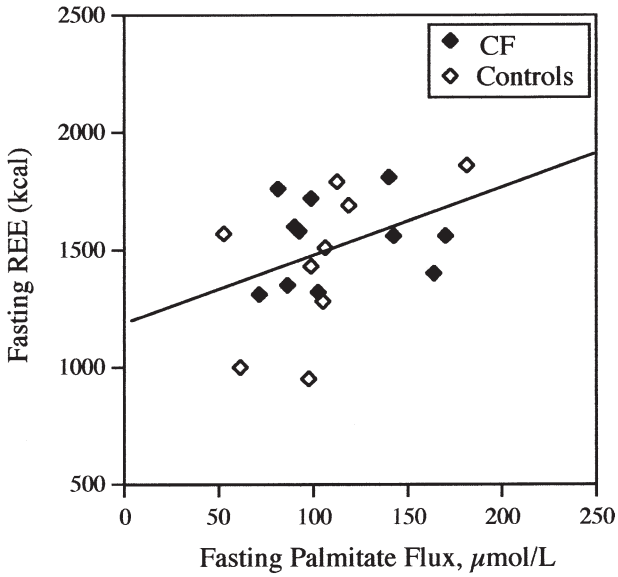

Fig 2. Basal palmitate flux is plotted *v* REE for each study participant, *r* = 0.39, *P* = .08.

While palmitate flux significantly decreased in response to insulin in both groups ($P < .001$), suppression was less in CF since the mean palmitate flux was 33% greater in CF compared to control subjects: CF = 32 ± 5 (26, 17 to 66), control = 24 ± 2 (23, 17 to 34) μmol/min; $P = .20$ (Fig 3B). There was considerably greater heterogeneity in insulin-induced suppression of plasma palmitate concentration and flux in CF patients compared to normal control subjects.

Energy Expenditure and Respiratory Quotient

REE did not differ between CF patients and control subjects at baseline (CF = 1,543 ± 178, control = 1,453 ± 324, $P = .4$) or after insulin infusion (CF = 1,550 ± 224, control = 1,572 ± 323, $P = .8$), nor within each group was there a significant change in REE in response to insulin. The respiratory quotient did not differ significantly between CF patients and control subjects at baseline (CF = 0.89 ± 0.04, control = 0.85 ± 0.02, $P = .07$) or in response to insulin (CF = 0.97 ± 0.05, control = 0.93 ± 0.05, $P = .1$). In both groups the respiratory quotient significantly increased in response to insulin ($P < .001$), indicating increased glucose oxidation.

DISCUSSION

Based on previous findings of increased HGP in the CF population,[2,35,36] we hypothesized that clinically stable, glucose-intolerant CF patients would have high rates of basal FFA turnover and that they would be resistant to insulin's suppressive effect on adipose tissue lipolysis. We found that, on average, basal palmitate concentration and flux were normal, suggesting that insulin availability and sensitivity in the overnight postabsorptive state is adequate to regulate adipose tissue lipolysis. When insulin was raised to typical postprandial levels, however, we found a modest defect in the ability of insulin to suppress FFA concentrations and flux.

This study represents the first report of FFA turnover in CF. The pattern of normal basal FFA turnover but impaired suppression of lipolysis in the presence of elevated insulin levels is similar to what we have observed for protein and carbohydrate metabolism. We previously reported that while protein catabolism rates were normal in the overnight postabsorptive period in clinically stable adult CF patients with abnormal glucose tolerance,[11] physiologic suppression of protein breakdown did not occur when insulin was increased to postprandial levels. Carbohydrate metabolism is also known to be altered in CF, and we and others have shown that HGP does not appropriately suppress in response to insulin.[2,35,36] Thus, a consistent metabolic pattern appears to be present, characterized by normal baseline substrate metabolism but defective suppression of carbohydrate, protein, and lipid metabolism by postprandial levels of insulin. In slender young adults, insulin levels are ordinarily low during fasting and rise in response to food intake. The presence of elevated insulin levels in the fed state serves to suppress breakdown of endogenous carbohydrate, protein, and fat stores so that dietary fuel sources can be preferentially used. It appears that this important mechanism of sparing body substrate stores is defective in CF.

In healthy individuals, lipolysis is exquisitely sensitive to the inhibitory effect of even low concentrations of insulin.[13] Fat

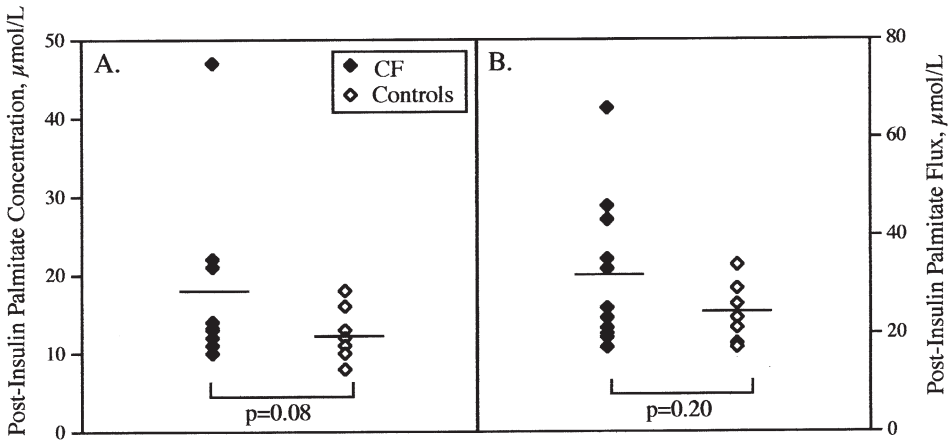

Fig 3. Palmitate concentration (A) and flux (B) in CF patients and control subjects during insulin infusion (0.5 mU/kg/min). The line indicates the median. Plasma insulin levels were 31 ± 3 μU/mL in CF patients and 32 ± 3 μU/mL in controls (mean ± SEM, *P* = .8).

mobilization is increased in states of insulin deficiency (type 1 diabetes),[25] insulin resistance (type 2 diabetes),[37] and sepsis.[38] During prolonged (4-day) fasting and in states of undernutrition, insulin suppresses proteolysis and HGP normally, but there is elevated resting lipolysis and marked resistance to the ability of insulin to suppress FFA release.[13,39] This serves to increase the availability of fatty acids as an energy source to spare body protein and carbohydrate stores. The CF pattern of substrate utilization is quite different than that seen with prolonged fasting, severe inflammation, obesity, or type 2 diabetes.

Oxidation of fatty acids provides energy for gluconeogenesis, and elevated fatty acid concentrations resistant to the suppressive effects of insulin are felt to contribute to excessive rates of gluconeogenesis and HGP in disease states.[40] Increased HGP generally results in hyperglycemia, as is the case in sepsis or diabetes. However, elevated HGP occurs in CF patients with normal fasting glucose levels, indicating that these patients metabolize the extra glucose being produced. Thus, increased HGP may be a metabolic adaptation to increased energy needs in CF. There may be deleterious consequences, however, if glucose is shunted away from other critical body pools. Gluconeogenesis is dependent both on a sufficient supply of key amino acids and on the availability of glycerol and FFA derived from lipolysis.[41] Lack of normal suppressibility of lipolysis and proteolysis by insulin in CF may provide the amino acid and fatty acid precursors necessary to fuel excessive HGP.

The volunteers with CF in the current study had insulin deficiency severe enough to cause IGT, but not so severe as to cause diabetes. Although they were not acutely ill, chronic subclinical infection and inflammation are present in most patients with CF. We have suggested that there may be a spectrum of metabolic derangement in CF, related to the degree of insulin deficiency and, probably more importantly, to the severity of the inflammatory process.[11] The CF clinic population at the University of Minnesota tends to be among the healthiest in North America, with better nutritional status and longer survival than average.[42] This allows us to study metabolism in CF patients under conditions where the confounding influences of malnutrition and acute illness are eliminated, and where one might expect the best possible CF outcomes. Even under these circumstances, however, significant metabolic abnormalities are found. The pattern we have described in this population—normal peripheral glucose utilization,[2] amino acid flux,[11] and FFA flux in the fasting state, but lack of normal suppression of HGP,[2] amino acid flux,[11] or fatty acid flux following insulin infusion—may represent the subtle metabolic consequences of mild insulin resistance related to chronic low-grade inflammation and infection. In the current study, CF patients had a considerably greater degree of heterogeneity in their response to the anti-lipolytic effects of insulin than control subjects. We suspect these differences were due to variable degrees of low-grade chronic inflammation, which, if present, were not sufficiently severe to affect REE. While the counter-regulatory hormone levels were normal, they would not be expected to demonstrate subtle inflammatory changes. Cytokine levels were not measured. Others have reported significant protein catabolism in the fasting state and impaired peripheral glucose utilization in CF patients considerably sicker than those we have studied.[12,43] Thus, more severe abnormalities in substrate utilization may represent the metabolic response to a greater degree of chronic inflammation. To the extent that more severe chronic inflammation can increase REE out of proportion to changes in body composition, future studies of lipolysis in CF or other inflammatory conditions should include measures of REE to avoid misinterpretation of the results.[28]

Until the last decade or so, essential fatty acid deficiency was common in CF and was believed to be related to severe malabsorption and malnutrition. Recently, however, as nutritional status has improved in CF, a specific essential fatty acid abnormality has been described even in well-nourished CF patients, consisting of low linoleic and docosahexaenoic acid levels in the presence of a relative excess of arachidonic acid levels.[44] This may represent a relative shunting of fatty acids toward proinflammatory pathways. Cholesterol levels are in general abnormally low in the CF population, while triglyceride levels are sometimes elevated, perhaps in response to inflammation.[45] How these patterns relate to the findings of the current study is unclear. We speculate however, that increased fatty acid availability may stimulate hepatic very-low-density lipoprotein synthesis, and contribute to the increased triglyceride levels seen in some CF patients.

In summary, we have shown defective insulin-induced suppression of lipolysis in clinically stable CF patients with IGT. This metabolic derangement is similar to what has been described in CF for amino acid and glucose metabolism, and suggests that even well-nourished, clinically stable CF patients with relatively modest abnormalities in insulin secretion experience abnormal substrate metabolism that may negatively impact their overall health and nutritional status.

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