

Hepatic Insulin Resistance and Defects in Substrate Utilization in Cystic Fibrosis

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Patients with cystic fibrosis (CF)-related diabetes (CFRD) have clinical features of both type 1 and type 2 diabetes. Past studies have documented peripheral insulin resistance in CF, and some studies have noted high hepatic glucose production (HGP) in CF patients. We hypothesized that patients with CF, similar to patients with type 2 diabetes, have hepatic insulin resistance. Cystic fibrosis is a catabolic condition, yet the etiology of catabolism is poorly understood. De novo lipogenesis is energy wasteful and precludes ketogenesis. Patients with CFRD rarely develop ketogenesis, despite insulin deficiency. We speculated that CF patients have de novo lipogenesis, and therefore evaluated substrate utilization in CF. Using [6,6-²H₂]glucose and a three-step hyperinsulinemic-euglycemic clamp, we measured HGP in 29 adult CF subjects and 18 control volunteers. Using indirect calorimetry, we measured lipid oxidation, oxidative glucose metabolism, and resting energy expenditure at baseline and at high levels of insulin. All subjects were characterized by oral glucose tolerance testing (OGTT) and National Diabetes Data Group criteria. The CF subjects had increased HGP when compared with control subjects (CF, 3.5 ± 0.6 ; control, 2.5 ± 0.5 mg · kg⁻¹ · h⁻¹; $P = 0.002$). Baseline HGP correlated with glucose levels obtained 2 h after a glucose load given for OGTT ($r = 0.69$, $P = 0.001$). Suppression of HGP by insulin was significantly less in all CF subgroups than in control subjects at peripheral insulin levels of 16 and 29 μ U/ml. At peripheral insulin levels of 100 μ U/ml and 198 μ U/ml, there was no difference in insulin suppression of HGP between CF and control subjects. At baseline, there was no significant difference between control and CF subjects for glucose or lipid oxidation. During maximum insulin stimulation, there was a greater tendency for nonoxidative glucose metabolism in all CF subjects. The CF subjects with abnormal glucose tolerance also had de novo lipogenesis. Our results indicate that CF patients have several defects in substrate utilization, including de novo lipogenesis. Furthermore, these results suggest that high hepatic glucose production and hepatic insulin resistance contribute to the high incidence of abnormal glucose tolerance in CF. *Diabetes* 48:1082–1087, 1999

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CF, cystic fibrosis; CFRD, cystic fibrosis-related diabetes; DCF, cystic fibrosis patients with diabetes; DEXA, dual X-ray absorptiometry; FBG, fasting blood glucose; GINF, glucose infusion; HGP, hepatic glucose production; IGTCF, cystic fibrosis patients with impaired glucose tolerance; NGTCF, cystic fibrosis patients with normal glucose tolerance; NPRQ, nonprotein respiratory quotient; REE, resting energy expenditure.

Cystic fibrosis (CF) patients have a higher incidence of diabetes than any other age-matched group (1). The type of diabetes is unique to CF and has features of both type 1 and type 2 diabetes.

Multiple studies have demonstrated that CF patients have decreased insulin secretion (2–4); several studies have also described peripheral insulin resistance in CF (5–7). Hepatic glucose production (HGP) is an important regulator of glycemic control (8), and several studies have demonstrated elevation of HGP in both type 1 (9) and type 2 (9,10) diabetes. Previous studies have also noted elevated HGP in CF (5,6,11). Insulin has a potent inhibitory effect on HGP that allows glycogen to be stored in the liver for use during fasting. Suppression of HGP by insulin is greatly reduced in type 2 diabetic patients (12) and in patients with liver disease (13). This condition has been termed “hepatic insulin resistance” (9) and is associated with peripheral insulin resistance (9,13,14). One previous study (6) describes both peripheral and hepatic insulin resistance in CF patients with frank diabetes; however, the same study describes resistance to insulin at the level of the liver but enhanced peripheral insulin sensitivity in CF patients who do not have diabetes. Due to these interesting findings, one purpose of our current study was to reexamine the relationship between hepatic and peripheral insulin sensitivity in CF.

Cystic fibrosis patients frequently have difficulty maintaining body weight, and the disease has been described as a catabolic condition (15,16). One mechanism of catabolism in CF is high resting energy expenditure (REE) (17,18), yet the etiology of high REE is not completely understood. Another clinical condition associated with both catabolism and high REE is HIV infection. One group (19) has described de novo lipogenesis, an energy wasteful process (20), in HIV-infected patients. We therefore wished to evaluate lipid oxidation in CF subjects using indirect calorimetry. We also utilized indirect calorimetry to evaluate oxidative and nonoxidative glucose metabolism.

RESEARCH DESIGN AND METHODS

Subject recruitment. Some 29 CF subjects, 17–37 years old, were recruited from the CF clinic at Texas Children's Hospital/Baylor College of Medicine. Patients were required to be medically stable at the time of the study (no hospital admissions for 6 weeks, no oral or intravenous antibiotics for 4 weeks preceding the study). Patients were excluded from participation if they had used oral or intravenous corticosteroid medications within 4 months of the study or if they had elevation of liver transaminases (SGOT, SGPT) on the most recent annual visit. Many subjects used low doses of inhaled corticosteroids; however, in proper doses, inhaled steroids do not cause endocrine changes (21–23). Patients were also excluded from participation if they were colonized with *Burkholderia cepacia* or were pregnant. All CF volunteers required replacement of pancreatic enzymes (exocrine insufficient) and all but one were weight-stable during the 3 months before the study. Eighteen normal

volunteers in good health were recruited by advertisement. They were matched to CF subjects for BMI, age, Tanner stage, and sex. None was an endurance-trained athlete, a physical state known to enhance insulin sensitivity (24), and none had an eating disorder as determined by history and physical examination. All subjects gave written informed consent as approved by the Institutional Review Boards at the University of Texas and Baylor College of Medicine.

Characterization of subjects

Oral glucose tolerance test. Two weeks to 1 day before the clamp study, each subject underwent a 3-h oral glucose tolerance test (OGTT). Subjects were categorized as having normal glucose tolerance (NGTGF), impaired glucose tolerance (IGTGF), or diabetes (DCF) according to standards set forth by the National Diabetes Data Group (25). The 2-h postprandial glucose levels and the fasting blood glucose (FBG) levels, used to determine if correlation existed between HGP and either 2-h postprandial glucose or FBG, were obtained from the OGTT in all subjects.

Dual X-ray absorptiometry. Body composition (lean body mass, fat mass) was measured on the day of the metabolic studies by dual X-ray absorptiometry (DEXA) (QDR 2000 Array Scan Mode; Hologic, Waltham, MA). This technique makes possible noninvasive assessment of both skeletal density and soft tissue composition with good precision. Total body scans require 10 min and have better than 1% precision for skeletal densities, better than 2% precision for lean tissue mass, and precision of 2–5% for calculation of percent fat (26). The correlation between DEXA and total-body potassium estimates of lean tissue is $r = -0.95$, and between DEXA and percent body fat estimate, $r = -0.9$ (27).

In vivo measurements

“Step-up” euglycemic-hyperinsulinemic clamp. Each subject spent the night before the study in the University of Texas Clinical Research Center. No patient was allowed to eat or drink after 8:00 P.M. Regular medications were withheld on the morning of the study, with the exception of inhaled respiratory treatments. Diabetic patients were given regular human insulin (Eli Lilly, Indianapolis, IN) subcutaneously throughout the night every 4 h as needed to maintain euglycemia (final dose given at 4:00 A.M.). On arrival at the unit, the patient's indwelling central venous catheter was accessed, or if the patient did not have an indwelling catheter, a 9-inch intracatheter (Becton Dickinson, Sandy, UT) was inserted into an antecubital vein. Isotonic saline was infused in the catheter at a low rate overnight. The next morning, an intravenous catheter was placed retrograde in the back of the opposite hand, and the hand was warmed with a heating pad to obtain arterialized blood samples.

To determine sensitivity of HGP to insulin, after the isotopic equilibration period (described below), subjects underwent a three-step hyperinsulinemic-euglycemic clamp (28). Regular human insulin was infused at rates of $10 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ in all subjects. After 90 min, the insulin dose was increased to 20 (20 CF and 11 control subjects [C]) or 40 (nine CF and seven C) $\text{mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$. After 90 min at this intermediate insulin dose, the insulin infusion was increased to a final step of $120 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ that lasted 120 min. Blood samples were taken at 5-min intervals for the immediate determination of plasma glucose using an automated glucose oxidase technique (Glucose Analyzer; YSI, Yellow Springs, OH). The results were used to titrate the infusion rate of 20% glucose as needed to maintain euglycemia. To prevent hypokalemia and hypophosphatemia, K_2HPO_4 was infused throughout the study. Glucose levels were clamped at $5.0 \pm 0.7 \text{ mmol/l}$ during all insulin doses of the clamp. Insulin levels were obtained at the end of each insulin dose and at baseline (before infusion of isotope).

Measurement of HGP. We measured HGP using a labeled glucose infusion (GINF) method (29). A primed (2.5 mg/kg) continuous ($2.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) infusion of $[\text{D-}6,6\text{-}^2\text{H}_2]\text{glucose}$ was administered for 2 h before the clamp (isotopic equilibration period) and throughout the infusion of insulin. Isotope was also added to the 20% dextrose infusion (used to maintain euglycemia during the clamp) in an amount calculated to yield a $[\text{D-}6,6\text{-}^2\text{H}_2]\text{glucose}$ isotopic enrichment approximately equal to that in plasma at isotopic equilibrium. This technique, developed by Finegood et al. (29), allows a relatively constant specific activity of isotope throughout the duration of the clamp. Arterialized blood was obtained before iso-

tope infusion, after the 2-h isotopic equilibration period, and 90 min after the initiation of each insulin step. Three blood samples were obtained in triplicate at 5-min intervals for each time point. Blood samples were separated, and the serum was frozen for future determination of plasma $[\text{D-}6,6\text{-}^2\text{H}_2]\text{glucose}$ enrichment.

HGP was calculated by subtracting the exogenous glucose infusion rate (20% dextrose infused to maintain euglycemia during the clamp) from the total rate of isotope appearance in serum. The rate of appearance (R_a) of $[\text{D-}6,6\text{-}^2\text{H}_2]\text{glucose}$ was determined by $R_a = i(E_i/E_p - 1)$, where i is the infusion rate of tracer in micromoles per kilogram per hour; and includes tracer added to the 20% dextrose during the clamp; E_i is the isotopic abundance of tracer; and E_p is the isotopic abundance in plasma at isotopic plateau. Calculations were made using a mean of three measurements obtained during the final 15 min of each infusion period.

To determine an appropriate dose of tracer to be added to nonlabeled glucose infusion, we performed preliminary studies in six CF subjects. During these studies, we infused each subject with $[\text{D-}6,6\text{-}^2\text{H}_2]\text{glucose}$ as described above and insulin doses of 20 or $120 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ for 3 h. We measured triplicate plasma $[\text{D-}6,6\text{-}^2\text{H}_2]\text{glucose}$ and serum glucose and insulin levels at 30-min intervals. From our measurements of HGP, we were able to calculate a reasonable estimate for addition of isotope to the 20% dextrose. We also were able to demonstrate stability of $[\text{D-}6,6\text{-}^2\text{H}_2]$ flux beginning 60 min after insulin infusion.

Indirect calorimetry. To measure substrate oxidation and REE, each subject underwent hood indirect calorimetry (Metascope; Cybermedics, Denver, CO) for 30 min at baseline and again during the final 30 min at maximum glucose disposal (insulin dose $120 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$). Substrate calculations yielding information on glucose oxidation and lipid oxidation were performed as previously described (10,30). Nonoxidative glucose metabolism was determined by subtracting the rate of glucose oxidation from the maximum glucose disposal rate (31) obtained during the final 30 min of the high-dose insulin clamp.

Measurement of counterregulatory hormones. We measured morning cortisol levels from blood collected at 6:30–7:00 A.M., before the initiation of insulin infusion. Blood for measurement of glucagon and norepinephrine was collected at the same time. Each sample was collected in the appropriate laboratory tube, separated, and frozen for future analysis.

In vitro measurements

Determination of plasma isotope enrichment. Plasma samples were derivatized, and plasma $[\text{D-}6,6\text{-}^2\text{H}_2]\text{glucose}$ enrichment was measured by gas chromatography/mass spectrometry (Metabolic Solutions, Boston, MA). Results were reported as moles percent excess.

Measurement of insulin levels. Serum-free insulin levels were measured by radioimmunoassay using a double-antibody technique (32) (Coat-A-Count; Diagnostic Products, Los Angeles, CA). Results were reported as microunits per milliliter.

Measurement of cortisol, norepinephrine, and glucagon levels. Measurement of serum cortisol (morning cortisol), glucagon, and norepinephrine levels were done by Endocrine Sciences Laboratory (Calabasa, CA).

Statistical analysis. Results are reported as means \pm SD for each subject group and, when noted, for the entire CF group. Repeated-measures analysis of variance for intergroup comparisons was performed with Bonferroni correction. Student's t test was used for calculating significance of insulin, epinephrine, and glucagon levels. Statistical significance was determined by P values <0.05 .

RESULTS

We studied 29 CF and 18 control subjects. Table 1 summarizes the clinical characteristics of the CF subgroups and the control group. Of the CF subjects, 9 were diabetic (area under the curve [AUC] 841 ± 34), 9 had impaired glucose tolerance (AUC 522 ± 29), and 11 had normal glucose tolerance (AUC 373 ± 49). Fasting blood glucose levels for each group are listed in Table 1. The 2-h post-glucose load levels were as follows: NGTGF, 112 ± 14 ; IGTGF, 179 ± 18 ; and DCF, 317 ± 70 .

TABLE 1
Characteristics of subjects

Subjects	Age	Sex (M/F)	Lean body mass	HbA _{1c} (%)	FBG	BMI
Control	26 \pm 5	6/8	39.2 \pm 7.7	5.1 \pm 0.4	5.1 \pm 0.6	20.7 \pm 2.7
All CF	25 \pm 6	12/17	41.4 \pm 8.1	7.1 \pm 1.7*	5.9 \pm 0.7*	20.5 \pm 2.8
NGTGF	23 \pm 5	5/6	41.2 \pm 8.9	5.3 \pm 1.0	4.6 \pm 2.9	20.9 \pm 3.7
IGTGF	26 \pm 6	5/4	41.9 \pm 7.9	6.1 \pm 1.1*	5.7 \pm 1.2*†	19.8 \pm 1.7
DCF	26 \pm 6	3/6	43.9 \pm 8.1	9.6 \pm 2.7*†	7.0 \pm 1.9*†	21.3 \pm 3.1

Data are means \pm SD. *Significantly different from control subjects; †significantly different from NGTGF.

All diabetic subjects but one were previously known to our group and had been treated with insulin for at least 1 year. The patient who was previously not known as diabetic did not receive insulin before these studies. Control subjects were matched with the CF subjects for age, weight, lean body mass, and sex. All control volunteers had normal glucose tolerance (AUC 345 ± 132), and their 2-h postprandial glucose was 119 ± 17 .

Basal HGP was determined using a modified Steele equation (33) and was measured after 2 h of isotopic equilibration. Each CF subgroup had significantly higher rates of HGP than control subjects, and there was no significant difference in HGP between CF subgroups (Fig. 1 and Table 2). There was no significant correlation between basal HGP and FBG in either CF or control subjects. However, basal HGP correlated with glucose levels obtained 120 min after the carbohydrate load during the OGTT ($r = 0.69$; $P = 0.001$) (Fig. 2). A similar correlation was noted between 60-min glucose levels and basal HGP. Insulin's ability to suppress HGP was measured by a stepwise euglycemic-hyperinsulinemic clamp. At each insulin step, there was no significant difference between the peripheral insulin levels achieved by control subjects and all CF subgroups for a given insulin infusion rate. Insulin infusion resulted in decreased HGP in all CF subgroups and in control subjects; however, the degree of suppression obtained at similar serum insulin levels was significantly less in all CF subgroups than in control subjects during insulin infusion of 10 and 20 $\text{mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ (Table 2). Supraphysiologic doses of insulin (100 and 190 $\mu\text{U}/\text{ml}$) resulted in similar suppression of HGP in all CF subgroups and control subjects. We did not find statistically significant differences in insulin's ability to suppress HGP between CF subgroups. Results from the clamps at insulin doses of 10, 20, and 40 $\text{mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ are reported in Table 2.

Morning cortisol levels were not different between CF and control subjects (CF, 15 ± 4 ; C, 13 ± 3 $\mu\text{g}/\text{dl}$; NS). At baseline, glucagon levels were similar between control and CF subjects (CF, 70.3 ± 24.0 ; C, 62.5 ± 20 pg/ml ; NS), and there was no correlation between glucagon levels and HGP in either control or CF subjects. Baseline norepinephrine levels did not differ between control and CF subjects (CF, 246 ± 32 ; C, 221 ± 44 pg/ml ; NS). At maximum glucose disposal, there was no

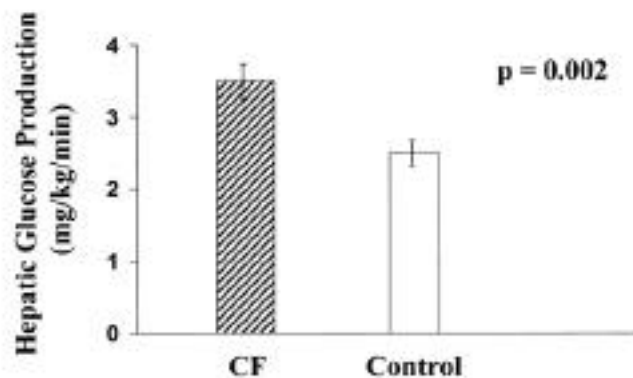


FIG. 1. Basal hepatic glucose production ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was measured after isotopic equilibration, and the results from all CF subgroups were averaged. There is a significant difference ($P < 0.05$) between the control subjects and the average from all CF subjects.

difference between control and CF subjects for either glucagon or norepinephrine levels.

Indirect calorimetry. As expected, REE was significantly higher in CF than in control subjects (CF, 1610 ± 292 ; C, 1295 ± 145 $\text{kcal}/24$ h; $P = 0.01$). At baseline, there was no significant difference in glucose oxidation between any CF subgroup and the control group. Although not significant, there was a tendency for the CF subjects to have higher lipid oxidation than the control subjects. At maximum glucose disposal, nonoxidative glucose metabolism, representing glycogen storage (34), was significantly lower in CF than in control subjects. Furthermore, the CF subjects demonstrated lipogenesis de novo. Indirect calorimetry data are given in Table 3. At baseline, the nonprotein respiratory quotient (NPRQ) was similar in control subjects and all CF subgroups (C, 0.91 ± 0.1 ; CF, 0.89 ± 0.1). After insulin stimulation, the NPRQ increased in all subjects, but was >1 in the CF subgroups (NGTCF, 1.0 ± 0.2 ; IGTCF, 1.1 ± 0.1 ; DCF, 1.1 ± 0.1 ; C, 0.9 ± 0.1). Differences between CF subgroups were not statistically significant.

DISCUSSION

Although previous studies have described high HGP in CF (5,6,11), this study is the first to correlate HGP with postprandial glucose levels and to report similarities in hepatic

TABLE 2
Insulin effects on hepatic glucose production

Time and HGP	Insulin dose ($\text{mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$)	Serum insulin level ($\mu\text{U}/\text{ml}$)	All CF	Control	NGTCF	IGTCF	DCF
Baseline							
HGP	No exogenous insulin	3 ± 1	$3.5 \pm 0.6^*$	2.5 ± 0.5	$3.2 \pm 0.2^*$	$3.7 \pm 0.7^*$	$3.4 \pm 0.6^*$
90 min	10						
HGP		16 ± 5	$2.3 \pm 0.7^*$	0.3 ± 0.7	$1.5 \pm 0.9^*$	$2.8 \pm 0.6^{*\dagger}$	$2.9 \pm 0.9^{*\dagger}$
% decrease			3.8 ± 1	88 ± 1	$53 \pm 6^*$	$26 \pm 2^{*\dagger}$	$40 \pm 4^{*\dagger}$
180 min	20						
HGP		29 ± 5	$0.2 \pm 0.1^*$	0.0 ± 0.1	$0.2 \pm 0.01^*$	$0.2 \pm 0.2^*$	$0.3 \pm 0.1^*$
% decrease			93 ± 1	100 ± 1	94 ± 1	94 ± 1	91 ± 1
300 min	40						
HGP		100 ± 7	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	0.1 ± 0.1
% decrease			99 ± 6	100 ± 0	100 ± 0	100 ± 0	99 ± 0.1

Data are means \pm SD. Percent decrease is relative to baseline HGP for each subgroup. *Significantly different from NGTCF; †significantly different from control subjects.

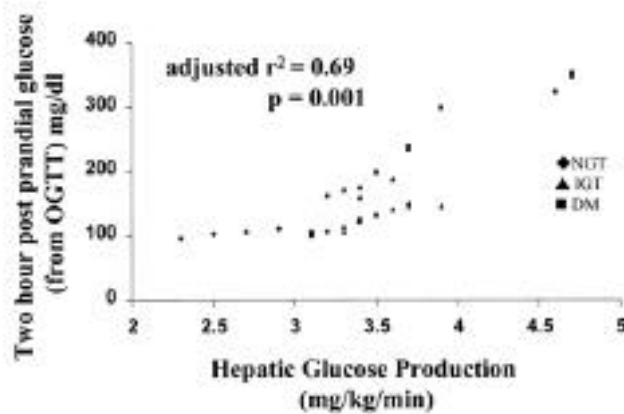


FIG. 2. The x-axis displays basal HGP measured at isotopic steady state. The y-axis denotes blood glucose levels obtained 2 h after a 75 mg/kg glucose load (maximum 75 mg) given for the oral glucose tolerance test. A strong correlation is noted.

insulin resistance in CF subjects with carefully defined differences in glucose tolerance. Furthermore, this study is the first to describe changes in substrate utilization in CF, particularly a tendency for de novo lipogenesis. Investigators documenting elevated HGP in type 2 diabetes suggest that the defect is genetic and is a primary contributor to the genesis of type 2 diabetes (9). At this time, the origin of elevated HGP in CF is unclear. Kien et al. (11) have generated the hypothesis that elevated basal HGP in CF is a compensatory mechanism for increased REE. That group found that elevated basal HGP disappeared when HGP was normalized for REE. Normalization of our basal HGP for REE finds similar loss of significance between control and CF subjects (data not shown). Moran et al. (6) postulated that elevated HGP may be related to enhanced gluconeogenesis, secondary to chronic elevation of catecholamines. Although our methods cannot distinguish gluconeogenesis from glycogenolysis, catecholamine levels did not differ between CF and control subjects.

We did not find significant differences in HGP between the CF subgroups. This similarity suggests that elevation of HGP is indeed a primary defect associated with CF. Although we normalized blood glucose levels in the DCF subgroup

before the clamp, the lack of difference in HGP in the CF subgroups could reflect suppression of HGP by chronic hyperglycemia for days preceding the study.

To evaluate hepatic insulin sensitivity, we used a labeled GINF method, analogous to the "hot GINF" method of Finegood (29), rather than a single-compartment model (35). The single-compartment model introduces two possible errors, one dependent on mixing volume and the other arising from the structure of the glucose system. Both errors are dependent on the plasma tracer specific activity (36). The inadequacy of this model for use during a hyperinsulinemic-euglycemic clamp has been well documented (37,38). Past studies (29,36) indicate that model errors can be minimized by reducing the rate of change of glucose specific activity during the non-steady state. We believe use of a modified hot-GINF technique (29) allowed us to most accurately measure HGP during the clamp. One weakness of our study is that we did not randomize the sequence of the insulin infusion dose. Because of the length of these studies, our results could be influenced by augmentation of existing glycogen stores from the glucose infusion, which could have the effect of sensitizing the liver to insulin and could explain the lack of differences in HGP suppression between CF subgroups at higher insulin doses. Despite this possible influence, our study clearly demonstrates differences in insulin's ability to suppress HGP between all CF subgroups and control volunteers at physiologic doses of insulin.

The only other study of hepatic insulin sensitivity in CF using similar methodology was done by Moran et al. (6). Both their study and ours describe elevated basal HGP and hepatic insulin resistance in CF subgroups with abnormal glucose tolerance. However, one of the most interesting differences between our current findings and those of Moran et al. is found in the subjects with abnormal glucose tolerance who do not have frank diabetes (EXO group in Moran et al., IGTCF group in our study). Moran et al. demonstrate hepatic insulin resistance, but enhanced peripheral insulin sensitivity, in this subgroup. In contrast, we have demonstrated both decreased peripheral (7) and decreased hepatic insulin sensitivity in subjects with impaired glucose tolerance. Austin et al. (5) have made a similar observation in this subgroup using a one-compartment model. The unique discordance between

TABLE 3
Substrate oxidation by indirect calorimetry

	NGTCF	IGTCF	DMCF	All CF	Control
Basal					
Glucose oxidation ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	2.4 ± 1.3	2.1 ± 1.0	1.9 ± 1.8	2.2 ± 1.2	2.4 ± 1.4
Lipid oxidation ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	0.58 ± 0.2	0.74 ± 0.5	0.72 ± 0.5	0.69 ± 0.4	0.47 ± 0.7
After insulin					
Glucose oxidation ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	4.3 ± 1.5	3.7 ± 1.0	3.2 ± 2.9	3.9 ± 1.2	4.3 ± 1.3
Lipid oxidation ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	0.07 ± 0.3	$-0.22 \pm 0.3^*$	$-0.12 \pm 0.7^*$	$-0.10 \pm 0.4^*$	0.02 ± 0.1
Nonoxidative glucose metabolism ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	$6.9 \pm 1.3^*$	$6.7 \pm 1.2^*$	$6.3 \pm 2.7^*$	$6.7 \pm 1.6^*$	9.7 ± 2.6
Glucose disposal rate ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	$10.8 \pm 1.0^*$	$10.3 \pm 1.0^*$	$10.3 \pm 2.5^*$	$10.5 \pm 1.5^*$	14.7 ± 2.4

Data are means \pm SD. *Significantly different from control subjects.

peripheral and hepatic insulin sensitivity as described (6) has not been previously reported in any other clinical condition. Although we have been unable to confirm these findings, differences in subject populations, such as worse pulmonary function and clinical status, may explain the findings.

Our group has reported insulin resistance (7) in CF subjects with normal glucose tolerance. Our current study reveals hepatic insulin resistance in this subgroup. Moran et al. (6) report normal hepatic, but enhanced peripheral, insulin sensitivity in normally glucose-tolerant subjects. One explanation for these findings may be that the subjects studied by Moran et al. were also pancreatic exocrine sufficient. We have previously reported (7) normal peripheral insulin sensitivity in three subjects with pancreatic exocrine sufficiency, and current evaluation of HGP in these three subjects reveals normal hepatic insulin sensitivity (data not shown). Patients with CF who are exocrine sufficient rarely develop CF-related diabetes (CFRD), thus these findings could reflect inherent differences in insulin action in exocrine-sufficient CF patients.

Although CF patients are treated with antibiotics associated with hepatotoxicity, such as semisynthetic penicillin derivatives and erythromycin, none of our subjects had elevated liver transaminases and none were being treated with ursodiolicholic acid. Thus, we do not believe that elevated HGP or hepatic insulin resistance is secondary to liver disease. Both insulin and glucagon regulate HGP (39,40), and insulin suppresses glucagon secretion (41). Previous investigators (4,42) have reported normal basal glucagon levels in CF. One study (4) noted decreased glucagon responsiveness to hypoglycemia and suggested that CF patients may have a defect in α -cell function. We found no differences in glucagon levels at baseline and maximum insulin infusion between control and CF subjects; however, we cannot completely rule out a role of glucagon affecting insulin suppressibility of HGP at physiologic insulin levels. Patients with CF frequently suffer from reactive airway disease, and many receive routine treatment with bronchodilators. Although bronchodilators are primarily β_2 agonists, they can have some β_1 effect, resulting in enhanced catecholamine secretion. Since there was no difference between the norepinephrine levels of control and CF subjects, it does not appear that our findings are related to bronchodilator therapy. Elevated cortisol levels can cause decreased insulin sensitivity (43) and could likely affect HGP. We did not find a difference in cortisol levels in CF and control subjects. However, it is possible that CF patients may have different diurnal variations of cortisol secretion than the control subjects. Future analysis using 24 h urine free cortisol measurements or obtaining cortisol levels after cortrosyn stimulation would be helpful to completely rule out a contributing role for cortisol in the abnormal glucose metabolism found in CF.

Early studies in type 2 diabetes (9) report correlation between HGP and fasting hyperglycemia; however, more recent studies (44) have not found this correlation and suggest (45) that the early reports documenting elevated HGP in type 2 diabetes are inaccurate secondary to one-compartment model errors. Our study did not reveal correlation between fasting blood glucose levels (obtained at OGTT) and HGP; however, we observed a correlation between HGP and 120-min glucose values obtained during an OGTT done 24 h before the clamp studies. Multiple studies (2-4) have documented decreased first-phase insulin response in CF

patients, and it is possible that the correlation between HGP and glucose levels exists secondary to decreased insulin secretion. However, this finding could also be secondary to failure of insulin to normally suppress HGP. Cystic fibrosis patients have a very high incidence of diabetes. We believe that failure of insulin to maximally suppress HGP causes high postprandial glucose levels and contributes to the high incidence of abnormal glucose tolerance found in CF.

Each of our subjects underwent indirect calorimetry for measurement of substrate utilization. The most interesting findings from these studies include lower rates of glycogen storage and net whole-body lipogenesis at maximum glucose disposal. One group (10) has described increased lipid oxidation in obese subjects both with and without diabetes. Interestingly, they also describe decreased nonoxidative glucose metabolism in obesity, but normal glycogen storage in obese type 2 diabetics. These authors suggest a contributory role of lipid oxidation to the defects of glucose metabolism reported in type 2 diabetes. Our current studies in CF describe decreased nonoxidative glucose utilization in all CF subjects, even those with impaired glucose tolerance. Thus it does not appear that substrate oxidation plays the same role in CFRD as it does in type 2 diabetes.

One explanation for the differences in glycogen storage between CF and control subjects could be the high rates of HGP found in CF. Lower rates of nonoxidative glucose metabolism could be one explanation for the hypoglycemia-like symptoms reported by many CF patients. This finding could represent a metabolic defect inherent in CF, or perhaps may represent subclinical liver disease.

Although indirect calorimetry has several limitations as an investigative tool (30), it has been used in multiple studies to describe lipid oxidation and has been used to describe de novo lipogenesis. Despite limitations, our results clearly describe a tendency for CF patients to have whole-body lipogenesis at maximum glucose utilization. Patients with CFRD generally do not develop ketoacidosis. Lipogenesis despite starvation would explain lack of ketosis in these patients.

We only found whole-body lipogenesis in the CF subjects with abnormal glucose tolerance. These findings suggest that in CF, de novo lipogenesis is related to carbohydrate utilization.

De novo lipogenesis has been reported in HIV-infected patients (19). Lipogenesis de novo is energy wasteful (20) and could potentially contribute to the greater need for calories in both of these conditions; however, at this time it is unclear whether the metabolic cause of lipogenesis, or its clinical significance, is similar in AIDS and CF.

At baseline, we note a tendency for the CF subjects to have higher lipid oxidation than control subjects. A similar tendency was reported by Mulligan et al. (46) in AIDS patients. The significance of this finding is not understood but may represent a metabolic abnormality that contributes to the tendency for lipogenesis de novo during insulin stimulation. Further studies using isotope methodologies would be helpful to determine the relationship of lipogenesis to substrate availability in CF.

Our current studies describe several important metabolic alterations in CF. We have found that CF patients have de novo lipogenesis, which could contribute to calorie wasting and lack of ketogenesis. They also have decreased glycogen storage, which may cause their reported symptoms of hypoglycemia. Previously, we postulated that a CF is caused by a com-

bined defect of insulin deficiency and insulin resistance (7). Our current studies also suggest that high hepatic glucose production and hepatic insulin resistance represent a third cause of the high incidence of abnormal glucose tolerance in CF.

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REFERENCES

- Finkelstein SM, Wielinski CL, Elliott GR, Warwick WJ, Barbosa J, Wu SC, Klein DJ: Diabetes mellitus associated with cystic fibrosis. *J Pediatr* 112:373-377, 1988
- Andersen O, Garne S, Heilmann C, Petersen KE, Petersen W, Koch C: Glucose tolerance and insulin receptor binding to monocytes and erythrocytes in patients with cystic fibrosis. *Acta Paediatr Scand* 77:67-71, 1988
- Wilmschurst EG, Soeldner S, Holsclaw DS, Kaufmann RL, Shwachman H, Aoki TT, Gleason RE: Endogenous and exogenous insulin responses in patients with cystic fibrosis. *Pediatrics* 55:75-82, 1975
- Moran A, Diem P, Klein D, Levitt MD, Robertson RP: Pancreatic endocrine function in cystic fibrosis. *J Pediatr* 118:715-723, 1991
- Austin A, Kalhan SC, Orenstein D, Nixon P, Arslanian S: Roles of insulin resistance and β -cell dysfunction in the pathogenesis of glucose intolerance in cystic fibrosis. *J Clin Endocrinol Metab* 79:80-85, 1994
- Moran A, Pyzdrowski KL, Weinreb J, Kahn BB, Smith SA, Adams KS, Seaquist ER: Insulin sensitivity in cystic fibrosis. *Diabetes* 43:1020-1026, 1994
- Hardin DS, LeBlanc A, Lukenbaugh S, Seilheimer DK: Insulin resistance is associated with decreased clinical status in cystic fibrosis. *J Pediatr* 6:948-956, 1997
- DeFronzo RA, Ferrannini E, Simonson DC: Fasting hyperglycemia in non-insulin-dependent-diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism* 38:387-395, 1989
- DeFronzo RA, Simonson D, Ferrannini E: Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 23:313-319, 1982
- Felber JP, Ferrannini E, Golay A, Meyer HU, Theibaud D, Curchod B, Maeder E, Jequier E, DeFronzo RA: Role of lipid oxidation in pathogenesis of insulin resistance of obesity and type II diabetes. *Diabetes* 36:1341-1350, 1987
- Kien CL, Horswill CA, Zipf WB, McCoy KS, O'Dorisio T: Elevated hepatic glucose production in children with cystic fibrosis. *Pediatr Res* 37:600-605, 1995
- Theibaud D, Jacot E, DeFronzo RA, Maeder E, Jequier E, Felber J: The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes* 31:957-963, 1982
- Marchesini G, Pacini G, Bianchi GP, Patrono D, Cobelli C: Glucose disposal, beta-cell secretion and hepatic insulin extraction in cirrhosis: a minimal model assessment. *Gastroenterology* 99:1715-1722, 1990
- Collins JR, Crofford OB: Glucose intolerance and insulin resistance in patients with liver disease. *Arch Intern Med* 124:142-148, 1969
- Shepherd R, Cooksley WG, Cooke WD: Improved growth and clinical, nutritional, and respiratory changes in response to nutritional therapy in cystic fibrosis. *J Pediatr* 97:351-357, 1980
- Hardin DS, LeBlanc A, Lukenbaugh S, Para L, Seilheimer DK: Proteolysis associated with insulin resistance in cystic fibrosis. *Pediatrics* 101:433-437, 1998
- Zemel BS, Kawchak DA, Cnaan A, Zhao H, Scanlin TF, Stallings VA: Prospective evaluation of resting energy expenditure, nutritional status, pulmonary function, and genotype in children with Cystic Fibrosis. *Pediatr Res* 40: 578-586, 1996
- Thomson MA, Wilmott RW, Wainwright C, Masters B, Francis PJ, Shepherd RW: Resting energy expenditure, pulmonary inflammation, and genotype in the early course of cystic fibrosis. *J Pediatr* 129:367-373, 1996
- Hellerstein MK, Grunfeld C, Wu K, Christiansen M, Kaempfer S, Kletke C, Shackleton CH: Increased de novo hepatic lipogenesis in human immunodeficiency virus infection. *J Clin Endocrinol Metab* 76:559-565, 1998
- Flatt JP: The biochemistry of energy expenditure. *Rec Adv Obes Res* 2:211, 1978
- Volovitz B, Amir J, Malik H, Kauschansky A, Varsano I: Growth and pituitary-adrenal function in children with severe asthma treated with inhaled budesonide. *N Engl J Med* 329:1703-1708, 1993
- Zeitlin S, Wood P, Evans A, Radford M: Overnight urine growth hormone, cortisol and adenosine 3' 5' cyclic monophosphate excretion in children with chronic asthma treated with inhaled beclomethasone dipropionate. *Respir Med* 87:445-448, 1993
- Kiviranta K, Turpeinen M: Effect of eight months of inhaled beclomethasone dipropionate and budesonide on carbohydrate metabolism in adults with asthma. *Thorax* 48:974-978, 1993
- Hardin DS, Azzarelli B, Edwards J, Wigglesworth J, Maianu L, Brechtel G, Johnson A, Baron A, Garvey WT: Mechanisms of enhanced insulin sensitivity in endurance-trained athletes. *J Clin Endocrinol Metab* 80:2437-2446, 1995
- National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039-1057, 1979
- Spector E, LeBlanc A, Shackelford L: Hologic QDR 2000 whole-body scans: a comparison of three combinations of scan modes and analysis software. *Osteoporos Int* 5:440-445, 1995
- Jensen MD, Kanaley JA, Roust LR, O'Brien PC, Braun JS, Dunn WL, Wahner HW: Assessment of body composition with use of dual-energy X-ray absorptiometry: evaluation and comparison with other methods. *Mayo Clin Proc* 68:867-873, 1993
- DeFronzo RA, Jacot E, Jequier E, Maeder E, Feller JP: The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 30:1000-1007, 1981
- Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps. *Diabetes* 36:914-924, 1987
- Simonson DC, DeFronzo RA: Indirect calorimetry: methodological and interpretative problems. *Am J Physiol* 258:E399-E412, 1990
- Golay A, DeFronzo RA, Ferrannini E, Simonson DC, Thorin D, Acheson K, Theibaud D, Curchod B, Jequier E, Felber JP: Oxidative and non-oxidative glucose metabolism in non-obese type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 31:585-591, 1988
- Morgan CR, Lazzaro A: Immunoassay of insulin: two antibody system: plasma insulin levels in normal, subdiabetic and diabetic rats. *Diabetes* 12:115-126, 1963
- Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann N Y Acad Sci* 82:420-430, 1959
- Meyer HU, Curchod B, Maeder E, Pahud P, Jequier E, Felber P: Modifications of glucose storage and oxidation in non-obese diabetics, measured by continuous indirect calorimetry. *Diabetes* 29:752-756, 1980
- Ferrannini E, Smith JD, Cobelli C, Toffolo G, Pilo A, DeFronzo R: Effect of insulin on the distribution and disposition of glucose in man. *J Clin Invest* 76:357-364, 1985
- Cobelli C, Bier DM, Ferrannini E: Modeling glucose metabolism in man: theory and practice. *Horm Metab Res* 24 (Suppl.):1-10, 1990
- Rizza RA, Mandarino LJ, Gerich JE: Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol* 240:E630-E639, 1981
- Wolfe RR: *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York, Wiley-Liss, 1992, p. 283-315
- Rao RH: Adaptations in glucose homeostasis during chronic nutritional deprivation in rats: hepatic resistance to both insulin and glucagon. *Metabolism* 44:817-824, 1995
- Lewis GF, Zinman B, Groenewoud Y, Vranic M, Giacca A: Hepatic glucose production is regulated both by direct hepatic and extrahepatic effects of insulin in humans. *Diabetes* 45:454-462, 1996
- Stevenson RW, Williams PE, Cherrington AD: role of glucagon suppression on gluconeogenesis during insulin treatment of the conscious diabetic dog. *Diabetologia* 30:782-790, 1987
- Lanng S: Glucose intolerance in cystic fibrosis. *Dan Med Bull* 44:23-39, 1997
- Plat L, Byrne MM, Sturis J, Polonsky KS, Mockel J, Fery F, Van Cauter E: Effects of morning cortisol elevation on insulin secretion and glucose regulation in humans. *Am J Physiol* 270:E36-E42, 1996
- Hother-Nielsen O, Beck-Nielsen H: Insulin resistance but normal basal rates of glucose production in patients with newly diagnosed mild diabetes mellitus. *Acta Endocrinol* 124:637-645, 1991
- Beck-Nielsen H, Hother-Nielsen O, Vaag A, Alford F: Pathogenesis of type-2 (non-insulin-dependent) diabetes mellitus: the role of skeletal muscle glucose uptake and hepatic glucose production in the development of hyperglycaemia. A critical comment. *Diabetologia* 37:217-221, 1994
- Mulligan K, Grunfeld C, Hellerstein MK, Neese RA, Schambelan M: Anabolic effects of recombinant human growth hormone in patients with wasting associated with human immunodeficiency virus infection. *J Clin Endocrinol Metab* 77:956-962, 1993