

Energy Expenditure and Genotype of Children with Cystic Fibrosis

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ABSTRACT. Increased energy expenditure, poor dietary intake, and fat malabsorption in patients with cystic fibrosis (CF) frequently lead to growth failure and malnutrition, which are associated with pulmonary failure and decreased survival. The study purpose was to understand better the energy expenditure and requirements in the mild pulmonary disease state in children. Resting and total energy expenditure were measured in 6- to 9-yr-old, pancreatic-insufficient children with CF ($n = 25$) and control children ($n = 25$) of similar age, gender, and weight. The effect of the most common genotype, homozygous $\Delta F508$, on energy expenditure was also investigated. Dietary intake, degree of fat malabsorption, body composition, physical activity, and clinical status were determined. The CF group had a 9% increase in resting energy expenditure, which was not related to genotype or severity of lung disease. Both CF genotype subgroups ($\Delta F508$ homozygous and all others) had a similar, modest resting energy expenditure increase. Total energy expenditure was increased by 12% in the entire CF group and by 23% in the $\Delta F508$ homozygous CF subgroup compared with controls. The total energy expenditure increase in $\Delta F508$ homozygous children may be related to increased voluntary physical activity, reflecting no activity reduction associated with lung disease, or to an unidentified genotype-related mechanism. The clinical implication is that a detailed physical activity assessment should be evaluated along with resting energy expenditure, either measured or estimated by equations, when daily energy needs are being determined for children with CF. (*Pediatr Res* 35: 451-460, 1994)

Abbreviations

CF, cystic fibrosis
REE, resting energy expenditure
TEE, total energy expenditure
FVC, forced vital capacity
FEV₁, forced expiratory volume in 1 s
FEF₂₅₋₇₅, forced expiratory flow between 0.25 and 0.75 s
²H, deuterium
¹⁸O, oxygen-18
BW, body weight

FFM, fat-free mass
SF, skinfold
TBW, total body water
FQ, food quotient
RQ, respiratory quotient
²H₂O, deuterium oxide
rCO₂, mean daily CO₂ production

CF, a common lethal genetic disorder with an autosomal recessive pattern of inheritance, is caused by mutations in the CF transmembrane conductance regulator gene (1-3). Generalized exocrine gland dysfunction results in abnormalities in several organ systems (4). In addition to the excessive losses of sodium chloride in sweat, abnormal mucus in the lungs contributes to the development of chronic obstruction, infection, and inflammation. Exocrine pancreatic insufficiency occurs in more than 85% of patients with CF, and, despite enzyme replacement therapy, malabsorption of fat and micronutrients may occur. According to a 1990 CF Foundation report, about 50% of patients cared for in the 114 organized CF centers had growth failure, with weight and/or height less than the 10th percentile for age and gender (5). CF-related growth failure is often attributed to chronic lung disease, although this causal relationship is not established.

Malnutrition may be caused by inadequate caloric intake, increased energy requirements, or both. The cause of the inadequate caloric intake is multifactorial, partly resulting from anorexia associated with lung infection and gastrointestinal discomfort associated with pancreatic insufficiency. Ingestion of multiple medications further interferes with appetite and food intake. Nutrient absorption, particularly fat, usually is decreased despite pancreatic enzyme supplementation. Energy expenditure may be increased as a result of chronic lung infection, fever, bronchodilator medications, a theorized increased work of breathing, and a possible basic molecular defect. The resulting negative energy balance depletes the body of its energy stores and impedes normal growth. Pencharz *et al.* (6) proposed that a vicious cycle is created in which the malnourished state places the child at risk for impoverished health because of lowered immune function, increased susceptibility to infections, continued anorexia, and inadequate food intake. The resulting catabolic state may be manifested by loss of skeletal mass, including respiratory muscles, and a decrease of lung elasticity, which further compromise the pulmonary system.

Energy expenditure in patients with CF has been previously investigated. Vaisman *et al.* (7) and Buchdahl *et al.* (8) documented an increase in REE in heterogeneous groups of patients with CF. TEE, using the doubly labeled water method, was

Received July 28, 1993; accepted November 23, 1993.

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Supported by the Cystic Fibrosis Foundation, Heinz Nutrition Center of The Children's Hospital of Philadelphia, and the National Institutes of Health Grant RR-00240 (General Clinic Research Center). Work performed in partial fulfillment of requirements for the degree of doctor of philosophy at the Pennsylvania State University (J.L.T.).

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reported by Shepherd *et al.* (9) to be increased in clinically well infants and toddlers with CF. In contrast, Spicher *et al.* (10), using a cumulative heart rate monitor method, found TEE to be equivalent between CF and healthy subjects despite increased REE in the CF group.

Contrasting findings exist regarding the pattern of energy expenditure among patients with the various genotypes of CF. Fried *et al.* (11) found that in normally nourished men and boys with CF and good pulmonary function no association existed between REE and the $\Delta F508$ homozygous genotype. Conversely, O'Rawe *et al.* (12) found that 7- to 15-yr-old subjects homozygous for $\Delta F508$ had higher REE compared with $\Delta F508$ heterozygous subjects or subjects with other mutations.

This study was designed to evaluate energy expenditure in a homogeneous prepubertal CF group who had pancreatic insufficiency, were well nourished with normal growth and body composition, and had mild pulmonary disease. This study avoids confounding variables such as poor lung function, malnutrition, and variable stages of pubertal development. The study hypotheses were as follows: 1) REE and TEE are higher in children with CF as compared with similar control children and 2) energy expenditure is related to CF genotype.

MATERIALS AND METHODS

Patients. Twenty-five prepubertal patients with CF were recruited from the CF center at The Children's Hospital of Philadelphia. Children were diagnosed with CF on the basis of clinical symptoms and duplicate quantitative pilocarpine iontophoresis sweat tests with sodium and chloride values greater than 60 $\text{mEq} \cdot \text{l}^{-1}$. All children were determined to be pancreatic insufficient on the basis of fecal fat analysis of a 72-h stool collection with 90% or less fat absorption, a stool trypsin value of less than 80 $\mu\text{m} \cdot \text{g}^{-1}$, or both. Selection criteria for patients with CF were defined as follows: 1) 5 to 10 years of age, 2) no hospitalization 6 wk before the study, 3) no acute pulmonary exacerbation or other acute illness at the time of data collection, and 4) no other disease or prescribed medication known to affect growth. Study data were collected at the time of the routine annual comprehensive examination.

Twenty-five healthy children from hospital and community sources were recruited as control subjects. The enrollment of these children began after approximately one half of the CF group had been evaluated. The group of children with CF provided selection criteria for controls to ensure comparable age, gender, and weight. General selection criteria for control subjects were as follows: 1) 5 to 10 years of age, 2) a weight range of 16 to 34 kg, 3) no known chronic or acute illness at the time of data collection, and 4) no other disease or prescribed medication known to affect growth. Individual matching of CF and control subjects was not carried out.

The study was conducted in the Nutrition and Growth Laboratory and the General Clinical Research Center of The Children's Hospital of Philadelphia during an 18-h overnight admission. Both parents and children gave informed written consent, and the protocol was approved by the Committee for the Protection of Human Subjects.

Growth and anthropometry. Weight, height, and SF thicknesses (triceps, biceps, subscapular, and suprailiac) were measured by one observer (J.T.) with standard methods as previously described (13, 14). Weight and height values were compared with National Center for Health Statistics reference standards (15), and percentiles and z scores were determined (Centers for Disease Control Anthropometric Software Program, Version 3.1, 1988, Division of Nutrition, Centers for Disease Control, Atlanta, GA). Ideal BW was determined as the weight that corresponded to the weight for age percentile equal to the actual height for age percentile on the National Center for Health Statistics growth chart (16). Percentage body fat was calculated using the triceps and subscapular SF in the prepubertal prediction equations of

Slaughter *et al.* (17), which are adjusted for body density, body water, and bone density. FFM was determined using TBW values from $^2\text{H}_2\text{O}$ dilution and assuming a 75.3% hydration factor of FFM in prepubertal children (18, 19).

Clinical status assessment. Clinical assessment of the patients with CF was based on consensus chest roentgenogram scores determined by two pediatric pulmonary physicians using the scoring system as described by Brasfield *et al.* (20). Clinical symptoms were rated by scores as described by Shwachman and Kulczycki (21). Pulmonary disease was evaluated at the time of the study in all patients with CF with standard pulmonary function methods for spirometry and plethysmography and compared with appropriate reference values (22, 23). These entities included FVC, FEV₁, FEF₂₅₋₇₅, and residual volume.

Three-day, weighed food records were collected at home as previously described (13). Food intake data were analyzed for 20 nutrients (Food Processor II Program-ESHA Research, Salem, OR). The home-based 72-h stool collection was begun on the 2nd d of the 3-d food record. Fecal fat was analyzed by the van de Kamer method (24) (Hahnemann University Hospital, Philadelphia, PA) or by a gravimetric method (Mayo Medical Laboratories, Rochester, MN). The coefficient of fat absorption was determined from fecal fat and dietary fat intake (25).

REE. REE was measured by open-circuit indirect calorimetry. In preparation, each child was admitted the preceding afternoon and received an evening meal that was standardized for calories, carbohydrate, protein, and fat. Each child fasted from food and medication for 12–14 h before REE testing. In the early morning the child was awakened, an axillary temperature was recorded, and physical activity was restricted.

A computerized metabolic cart (Gould 2900 or SensorMedic 2900 Z, SensorMedics, Yorba Linda, CA) was used to measure REE for 60 min in a quiet, thermoneutral room. A large, clear ventilated hood for sampling gases was placed over the head of the subject while he or she was resting in a supine position. The modified equation as described by Weir (26) was used to calculate energy equivalency from the oxygen consumption and carbon dioxide production. The first 10 min were devoted to environmental adjustment by the child and were eliminated from calculations. REE data were compared with predicted values from World Health Organization basal metabolic rate equations (27).

TEE and TBW. TEE was measured by the doubly labeled water method. The $^2\text{H}_2\text{O}$ dose was 0.14 g $^2\text{H}_2\text{O} \cdot \text{kg}$ estimated TBW^{-1} (28) and was 99.8 atom percent deuterium (Aldrich, Milwaukee, WI). The dose of ^{18}O in water was 0.3 g $\text{H}_2^{18}\text{O} \cdot \text{kg}$ estimated TBW^{-1} (28) and ranged from 14 to 15% ^{18}O (EG & G Mound, Miamisburg, OH).

On the day of admission, fasting began at 1830 h, and a baseline urine sample was collected. At 2000 h, the isotopes were administered orally. Overnight urine and the first urine void of the morning (1) were collected and pooled to adjust for isotope losses. The second urine void of d 1 was collected to determine TBW ($^2\text{H}_2\text{O}$) and to begin the TEE metabolic period. At home, the first urine voids of d 4, 7, and 10 were collected. The subjects observed their typical daily routines in their usual environment. Urinary ^2H and ^{18}O isotopic abundances were measured by isotope ratio mass spectroscopy (Nuclide 6–60, State College, PA; Global Geochemistry Corporation, Canoga Park, CA). Urinary ^2H samples were analyzed in duplicate, with reproducibility of ± 5 parts per mille for isotopically enriched samples and ± 3 parts per mille for the unenriched baseline samples. One out of 10 ^{18}O samples were analyzed in duplicate, and reproducibility was ± 0.1 parts per mille.

Standard equations for calculation of TEE (18) were used, with adjustment for overnight loss after dosing: $\text{rCO}_2 = (\text{N}/2.078) (1.01 k_o - 1.04 k_H) - 0.0246 r_{\text{Gr}}$, where rCO_2 is mean daily CO_2 production, N is TBW, k_o and k_H are ^{18}O and ^2H isotope elimination rates, and r_{Gr} is the rate of water loss via fractionating gaseous routes. $r_{\text{Gr}} = 1.05 \text{N} (1.01 k_o - 1.04 k_H)$. The calculations assume that the ^2H and ^{18}O dilution spaces are

Table 1. Growth and body composition characteristics of children with CF and control children

	Cystic fibrosis (13F/12M)			Control (11F/14M)		
	Mean	SD	Range	Mean	SD	Range
Age (y)	7.7	1.3	6.1–9.5	7.6	1.1	5.7–10.1
Weight (kg)	23.9	4.4	16.5–34.0	24.8	3.7	17.2–33.1
Weight percentile	39	25	3–93	52	25	12–97
Height (cm)	123.2	8.3	109.9–139.4	125.4	8.9	107.6–144.2
Height percentile	38	23	7–90	54	26*	6–98
% Ideal BW	101	8	86–117	101	11	82–122
Triceps SF (mm)	9.0	2.3	4.8–15.9	10.2	2.6	5.8–17.3
Triceps z score	−0.42	0.59	−1.32–1.19	0.01	0.80*	−1.06–1.52
% Body fat–SF	13.6	3.3	5.5–22.7	15.3	2.9	9.9–22.5
% Body fat–TBW	13.0	6.3	2.1–24.2	17.4	5.2†	8.7–30.2
Upper arm circumference (cm)‡	18.9	1.9	14.9–22.4	19.5	1.5	16.5–22.5
Upper arm circumference z score‡	−0.15	0.80	−1.55–1.36	0.28	0.88	−1.21–1.77
FFM (kg SF)	20.6	3.6	15.6–29.9	21.0	3.2	14.6–28.1
FFM (kg TBW)	20.7	3.6	14.8–29.7	20.5	3.5	14.3–30.2

* Significant difference between groups by *t* test, $p < 0.05$.

† Significant difference between groups by *t* test, $p < 0.01$.

‡ $n = 24$ for CF group.

Table 2. Energy expenditure and dietary intake of children with CF and control children

	Cystic fibrosis				Control			
	<i>n</i>	Mean	SD	95% CI*	<i>n</i>	Mean	SD	95% CI*
REE (kcal·d ^{−1})	23	1193	120	1141–1245	23	1144	111	1096–1192
REE (% WHO, kcal·d ^{−1})	23	114	7	111–117	23	108	6†	105–111
REE (kcal – kg BW ^{−1} ·d ^{−1})	23	50	6	47–53	23	46	4†	44–48
REE (kcal – kg FFM – SF ^{−1} ·d ^{−1})	23	58	6	55–61	23	55	5‡	53–57
REE (kcal – kg FFM – TBW ^{−1} ·d ^{−1})	23	58	6	55–61	23	56	5	54–58
TEE (kcal·d ^{−1})	19	2012	435	1802–2222	23	1808	295	1680–1936
TEE (% WHO, kcal·d ^{−1})	19	105	22	94–116	23	96	12	91–101
TEE (kcal – kg BW ^{−1} ·d ^{−1})	19	84	17	76–92	23	75	10	71–79
TEE (% WHO, kcal – kg BW ^{−1} ·d ^{−1})	19	109	19	100–118	22	95	12†	90–100
TEE (kcal – kg FFM – SF ^{−1} ·d ^{−1})	19	97	19	88–106	23	88	12	83–93
TEE (kcal – kg FFM – TBW ^{−1} ·d ^{−1})	19	97	19	88–106	23	88	12	83–93
TEE/REE	18	1.68	0.31	1.53–1.83	22	1.61	0.21	1.52–1.70
TEE as % dietary intake (kcal·d ^{−1})	19	100	28	87–114	22	103	18	95–111
Intake (kcal·d ^{−1})	24	2060	447	1871–2249	24	1833	392	1167–1999
Intake (kcal·kg BW ^{−1} ·d ^{−1})	24	88	24	78–98	24	75	16	68–82
Intake (% 1989 RDA)	24	103	22	94–112	24	93	19	85–101
% kcal as fat	24	34	5	32–36	24	32	6	29–35

* CI, confidence interval.

† Significant difference between groups by *t* test, $p \leq 0.05$.

‡ Significant difference between groups by *t* test, $p \leq 0.01$.

larger than the body water pool, 4% and 1% respectively (18), and that fractionated water loss is about 23% of total water turnover (29). The elimination rates for ¹⁸O (k_O) and ²H (k_H) were determined with the two-point method. The 9-d metabolic period was defined as the difference between d 1 and d 10. The d 7 rCO₂ was used as a consistency check of d 10 rCO₂. If rCO₂ differed more than 10% between the 2 d, data for that subject were excluded because of unacceptable variability. RQ was estimated from the FQ (30), calculated from 3-d diet records during the metabolic period. In the patients with CF, the FQ was adjusted for the coefficient of fat absorption. TEE data were compared with World Health Organization recommended energy requirements for children (27). Factors affecting the TEE metabolic period were recorded, *i.e.* yearly season of metabolic period and prescribed use of bronchodilators that increase energy expenditure–heart rate. Documentation of actual bronchodilator compliance was not obtained.

Genotype analysis. Genomic DNA was isolated from blood samples (Applied Biosystems, Model 340A Nucleic Acid Extractor, Foster City, CA). The presence of the ΔF508 mutation was determined by allele-specific oligonucleotide hybridization as

described in Kerem *et al.* (3). Patients with CF were classified as ΔF508 homozygous, ΔF508 heterozygous, or having other mutations. Control subjects were not evaluated.

Estimates of physical activity. Parents estimated their child's physical activity each day during the metabolic period. A questionnaire adapted from Murphy *et al.* (31) provided a score for daily physical activity: sedentary = 1, slightly active = 2, moderately active = 3, and active = 4. Parents chose the category that best described the child's physical activity for each day, and the mode score for the 9-d metabolic period was used to classify the overall activity. The TEE/REE ratio, representing the energy used for physical activity, thermic effect of food, and growth, was also calculated.

Statistical analysis. Univariate comparisons of growth, body composition, dietary intake, and energy expenditure between the two groups of children were evaluated by examining means, SD, 95% confidence intervals, and range of values within each group. Unpaired *t* tests, with logarithm transforms when appropriate, were used to measure distribution differences between the two groups and to assess statistical significance. Additional univariate comparisons were performed by separating the children with CF

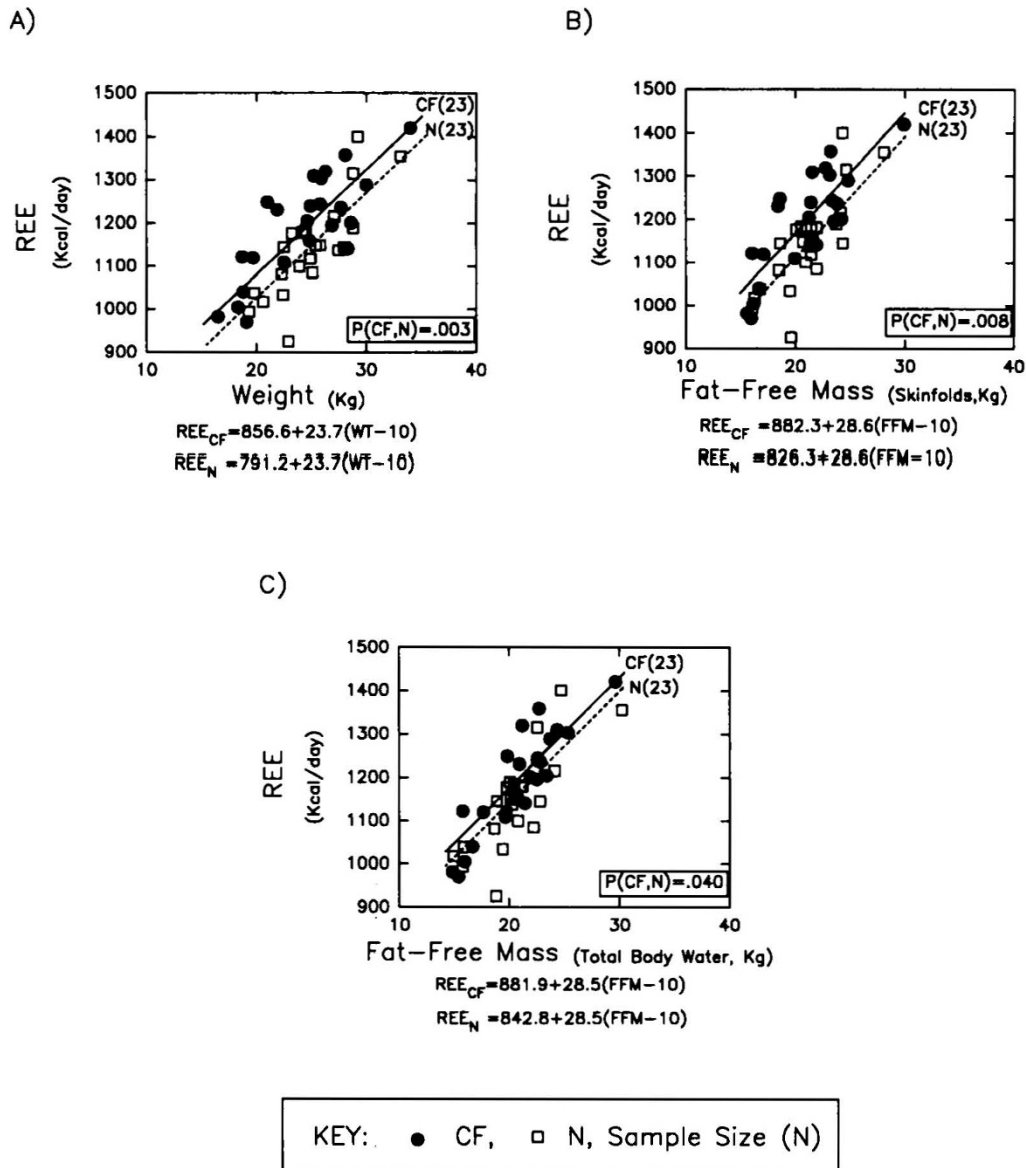


Fig. 1. Analysis of covariance of REE and BW (A), FFM from two SF measurements (B), and FFM from TBW (C) in children with CF (CF) and control children (N).

into two subgroups: homozygous $\Delta F508$ and all other genotypes. Univariate comparisons for ordinal scaled variables were accomplished with the Wilcoxon rank sum statistic, and categorical variables were determined by corrected χ^2 and Fisher's exact tests. All data analysis was performed with SAS (SAS Institute, Cary, NC), with a conservative two-tailed testing approach.

The primary analysis for energy expenditure differences of groups and subgroups was based on linear model adjustment using covariates known or suspected to influence energy expenditure. This analysis of covariance method accommodated adjustment for weight, gender, body composition measures of FFM, and genotype. It also allowed the interaction of these covariates to be evaluated and the validity of the model(s) to be assessed. A small bias may appear in these estimates with FFM adjustment because of the inherent variability associated with its estimation. Outliers in these models were identified by evaluating studentized residuals for each model. Sensitivity analyses were performed by deleting outlier observations, applying logarithm transforms, and estimating missing values by the method of Bartlett (32). Pulmonary function differences between the two subgroups of children with CF were evaluated with the linear model with adjustment for age, gender, and weight.

RESULTS

Growth and clinical characteristics. Growth and body composition characteristics for all subjects are presented in Table 1. Age, weight, and height were similar for the CF and control groups, which reflects the procedure used for selecting controls. However, mean height for age percentile was lower for the CF group. Nutritional status, as indicated by percentage ideal BW, was similar for both groups. Most body composition measures were comparable, with the exceptions of triceps z score and percentage body fat determined by TBW, both of which were lower for the CF group. Mean \pm SD (range) of pulmonary function, indicated by FEV₁ percentage predicted, was $89 \pm 16\%$ (61–117%), and the chest roentgenogram score was 21 ± 2 (16–24), out of a maximum score of 25. These measures indicated mild pulmonary disease for the children with CF. The CF clinical severity score was 89 ± 5 (78–97), which reflects excellent clinical status.

REE. The REE results of four subjects (two with CF and two control) were omitted because of equipment malfunction. REE values (Table 2) demonstrated higher means in children with CF, e.g. an 8.5% difference between mean values for kcal·kg·d

Table 3. Energy expenditure and dietary intake of children with CF, by genotype, and control children

	$\Delta F508/\Delta F508^*$				$\Delta F508/O$ and O/O^*				Control			
	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range	<i>n</i>	Mean	SD	Range
Age (y)	14	7.7	1.4	6.1–9.5	11	7.8	1.3	6.1–9.4	25	7.6	1.1	5.7–10.1
Weight (kg)	14	23.9	3.8	18.3–30.0	11	23.9	5.2	16.5–34	25	24.8	3.7	17.2–33.1
Weight percentile	14	41	25	12–93	11	37	27	3–89	25	52	25	12–97
Height percentile	14	39	24	11–90	11	37	23	7–71	25	54	26	6–98
% Ideal BW	14	100	9	86–117	11	102	7	88–112	25	101	11	82–122
FFM (kg TBW)	14	20.9	3.0	15.9–25.4	11	20.5	4.3	14.8–29.7	25	20.5	3.5	14.3–30.2
% Body fat – TBW	12	13.1	6.4	2.1–24.2	11	13.9	6.5	4.3–23.2	24	17.3	5.3	8.7–30.2
FEV ₁ (% predicted)	12	93	14	67–114	11	85	19	61–117				
	<i>n</i>	Mean	SD	CI†	<i>n</i>	Mean	SD	CI†	<i>n</i>	Mean	SD	CI†
REE (kcal·d ⁻¹)	13	1191	99	1131–1251	10	1197	148	1091–1303	23	1144	111	1096–1192
REE (% WHO, kcal·d ⁻¹)	13	114	7	110–118	10	115	7	110–120	23	108	6	105–111
REE (kcal·kg BW ⁻¹ ·d ⁻¹)	13	50	6	46–54	10	50	6	46–54	23	46	4	44–48
REE (kcal·kg FFM – TBW ⁻¹ ·d ⁻¹)	13	57	5	54–60	10	59	7	54–64	23	56	5	54–58
TEE (kcal·d ⁻¹)	10	2198	451	1875–2521	9	1806	324	1557–2055	23	1808	295	1680–1936
TEE (kcal·kg BW ⁻¹ ·d ⁻¹)	10	92	16	81–103	9	76	15	65–88	23	75	10	71–79
TEE (kcal·kg FFM – TBW ⁻¹ ·d ⁻¹)	10	104	12	95–113	9	89	15	77–100	23	88	12	83–93
TEE/REE ratio	10	1.85	0.26	1.66–2.04	8	1.46	0.21	1.28–1.64	22	1.61	0.21	1.52–1.7
Intake (kcal·d ⁻¹)	14	1983	46	1714–2252	10	2168	419	1868–2468	24	1833	392	1167–1999
Intake (kcal·kg BW·d ⁻¹)	14	84	19	73–95	10	93	29	72–114	24	75	16	68–82
Intake (% 1989 RDA)	14	100	23	87–113	10	108	21	93–123	24	93	19	85–101
% kcal as fat	14	32	5	29–35	10	36	5	32–40	24	32	6	29–35

* $\Delta F508/\Delta F508$ homozygous genotype; $\Delta F508/O$ heterozygous and unknown mutation; O/O unknown mutation.

† CI, confidence interval.

and 3.6% difference for kcal·kg·FFM·d⁻¹ by TBW (FFM-TBW).

Differences in REE between groups were evaluated adjusting for weight, FFM, and gender, all of which are factors known to influence REE. Using weight, FFM-SF, or FFM-TBW as covariates in three separate models (Fig. 1A–C) yielded greater CF group differences, compared with controls, of 65.4 kcal·d⁻¹ for weight ($p = 0.003$), 56.0 kcal·d⁻¹ for FFM-SF ($p = 0.008$), and 39.1 kcal·d⁻¹ for FFM-TBW ($p = 0.04$). Including gender (not shown) in the model with weight provided an adjusted group difference of 70.9 kcal·d⁻¹ ($p = 0.001$), with boys having higher REE in both groups.

Controlling for gender and FFM-SF provided an adjusted group difference of 60.6 kcal·d⁻¹ ($p = 0.003$). Gender was not an important factor for adjustment with FFM-TBW ($p = 0.14$). The influence of two control subject outliers (Fig. 1A) was investigated and did not alter results. In fact, analysis excluding the two outliers resulted in more significant p values for differences between groups, e.g. $p = 0.02$ with FFM-TBW (Fig. 1C).

Genotype classifications (Table 3) had 14 (56%) patients who were homozygous for the $\Delta F508$ mutation, 7 (28%) heterozygous ($\Delta F508$ /unknown), and 4 (16%) unknown mutations. These latter 11 patients were grouped together as the mixed heterozygous subgroup and compared with the 14 homozygous patients. Adjusting for weight and FFM separately yielded similar REE results for the two CF subgroups (Fig. 2A–C). The REE for both CF subgroups was significantly higher than the control group when the model was adjusted for either weight or FFM-SF. When FFM-TBW was used to adjust the model, the homozygous subgroup was not different from controls ($p = 0.18$). Estimated mean REE differences between the two CF subgroups were 5.2 kcal·d⁻¹ for weight adjustment, 7.0 kcal·d⁻¹ for FFM-SF adjustment, and 20.9 kcal·d⁻¹ for FFM-TBW adjustment. Including gender in the model with weight or with FFM-SF provided similar results. However, the model with gender and FFM-TBW (not shown) indicated no significant difference between the homozygous subgroup and controls despite a significant differ-

ence ($p = 0.04$) between the mixed heterozygous subgroup and controls.

TEE. The TEE results of eight subjects (six with CF and two control) were excluded because of unacceptably high variability in rCO₂. The CF group (Table 2) had 12% higher TEE expressed as kcal·kg body weight⁻¹·d⁻¹ compared with the control group, with 95% confidence intervals of 76 to 92 and 71 to 79, respectively. Mean differences between the TEE of CF and control groups were 9–12% less in the control group when TEE were expressed in other units.

Analysis of TEE as measured by kcal·d⁻¹ yielded a significant difference of 216 kcal·d⁻¹ ($p = 0.04$) between the CF and control groups when adjusted for weight (Fig. 3A). TEE analysis, adjusted for weight and gender (Fig. 3B) showed that boys had higher TEE, 234 kcal·d⁻¹, in both CF and control groups. When FFM-SF or FFM-TBW were used for adjustment (Fig. 3C and D), differences between the CF and control groups were smaller (191 kcal·d⁻¹ for FFM-SF and 156 kcal·d⁻¹ for FFM-TBW). Four outlier observations, three CF and one control, were identified (Fig. 3A). Deleting these observations resulted in higher p values: $p = 0.11$ (weight), $p = 0.17$ (FFM-SF), and $p = 0.16$ (FFM-TBW).

In marked contrast to the REE genotype analysis, the $\Delta F508$ homozygous children expended 21% and 23% more total daily energy (kcal·kg·d⁻¹) than the mixed heterozygous subgroup and the controls, respectively. TEE, adjusted for weight, resulted in a difference of 406 kcal·d⁻¹ ($p = 0.004$) between the $\Delta F508$ homozygous subgroup versus the mixed heterozygous subgroup and a difference of 408 kcal·d⁻¹ ($p = 0.001$) between the homozygous $\Delta F508$ subgroup versus the control group (Fig. 2D). In this model, a difference of only 2.7 kcal·d⁻¹ was observed between the mixed heterozygous subgroup and the control group. Removing the four outliers yielded smaller differences between the homozygous subgroup and mixed heterozygous ($p = 0.06$) and control ($p = 0.02$) subjects.

Food intake, lung function, and physical activity. For children with CF, caloric intake was 17% higher ($p = 0.04$) when kcal·kg·d⁻¹ were examined. Dietary intake was about 100% of TEE

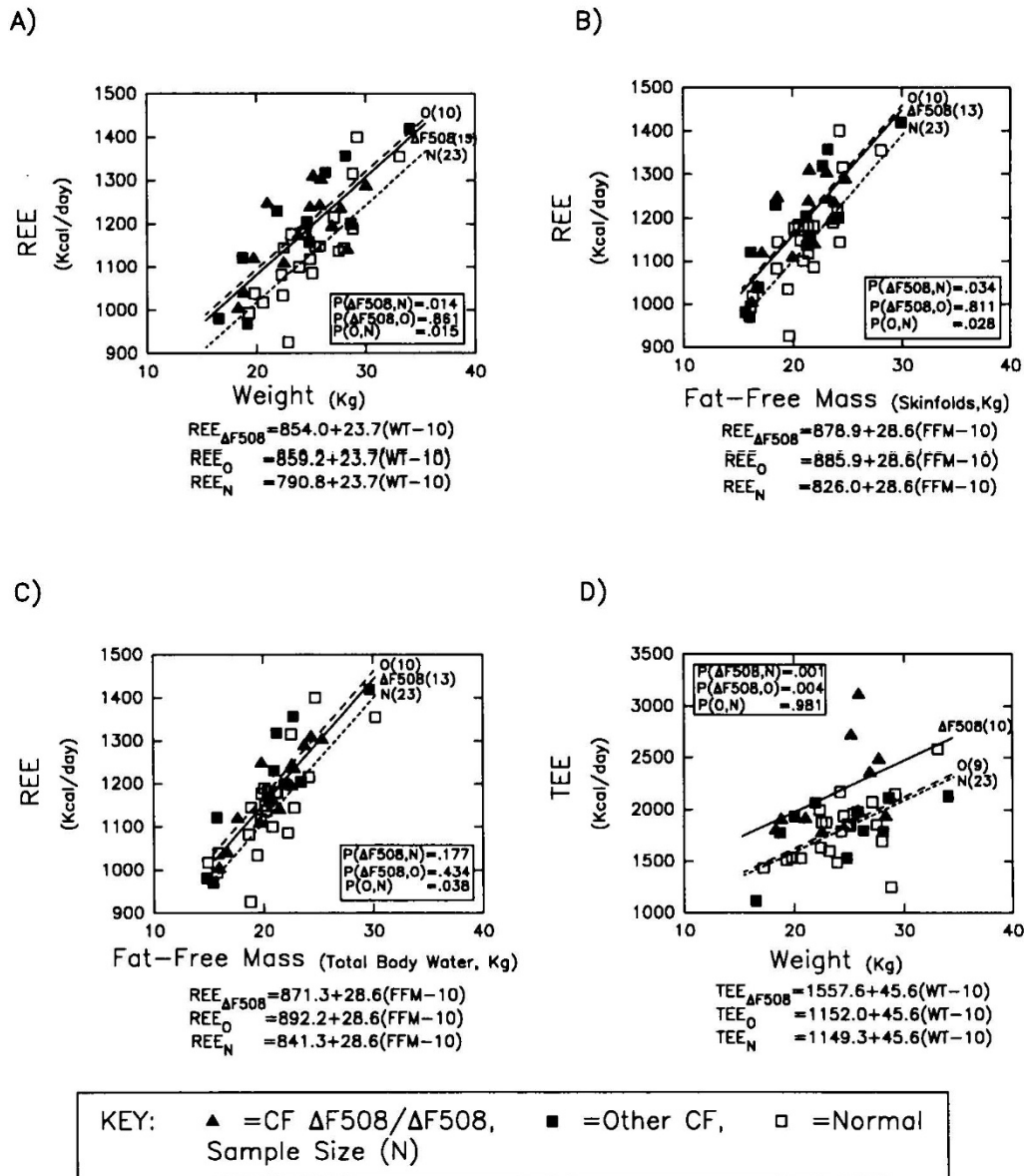


Fig. 2. Analysis of covariance of REE by weight and FFM presented by genotype category in children with CF and controls (A-C) and of TEE by weight presented by genotype category in children with CF (CF) and controls (N) (D).

in both groups. Small differences were found between the two genotype subgroups and the control group in caloric intake (Table 3). The mean coefficient of fat absorption from the stool analysis for 23 of the patients with CF was 87%, and the median value was 90%. FQ, calculated from TEE metabolic period diet records, was used to determine RQ for the calculation of TEE. RQ for patients with CF was adjusted for loss of stool fat with the coefficient of fat absorption. The mean RQ for the CF group was 0.88 before and after stool fat adjustment, and the unadjusted FQ, also 0.88, was used as the RQ for the control group.

The effect of lung disease on REE and TEE, as measured by FEV₁ and FVC% predicted, resulted in no significant associations. When genotype subgroup data were compared, including age, gender, and weight (either singly or combined) in the linear model, similar results were found. Patients with CF were examined for the effect of genotype ($\Delta F508$ homozygous subgroup versus mixed heterozygotes) on pulmonary function. Nonsignificant differences (mean \pm SD) were observed: FVC, 105 \pm 12% versus 99 \pm 12% predicted; FEV₁, 93 \pm 14% versus 85 \pm 19% predicted; FEV₁/FVC, 90 \pm 10% versus 85 \pm 16% predicted; FEF₂₅₋₇₅, 75 \pm 24% versus 72 \pm 38% predicted; and residual volume, 144 \pm 54% versus 178 \pm 48% predicted. The $\Delta F508$

homozygous subgroup tended to have better scores for these pulmonary measures.

TEE divided by REE is an indicator of physical activity and thermic effect of food during the 9-d metabolic period. Thermic effect of food was not measured and was assumed to be similar in all groups and subgroups. The TEE/REE ratios (Table 2) were not statistically different between the entire CF and control groups, adjusting for weight or FFM. However, a difference in the ratio was noted between the $\Delta F508$ homozygous and the mixed heterozygous subgroups (Table 3). The control group ratio, which was located between those of the two CF subgroups, was different from both. Physical activity scores from parental observations for the two genotype CF subgroups and the control group were compared with rank test procedures, and no differences were observed.

DISCUSSION

There were several salient findings in this study of adequately nourished, prepubertal, pancreatic-insufficient children with mild lung manifestations of CF. First, REE was increased 6–9% above controls and appeared independent of genotype subgroup

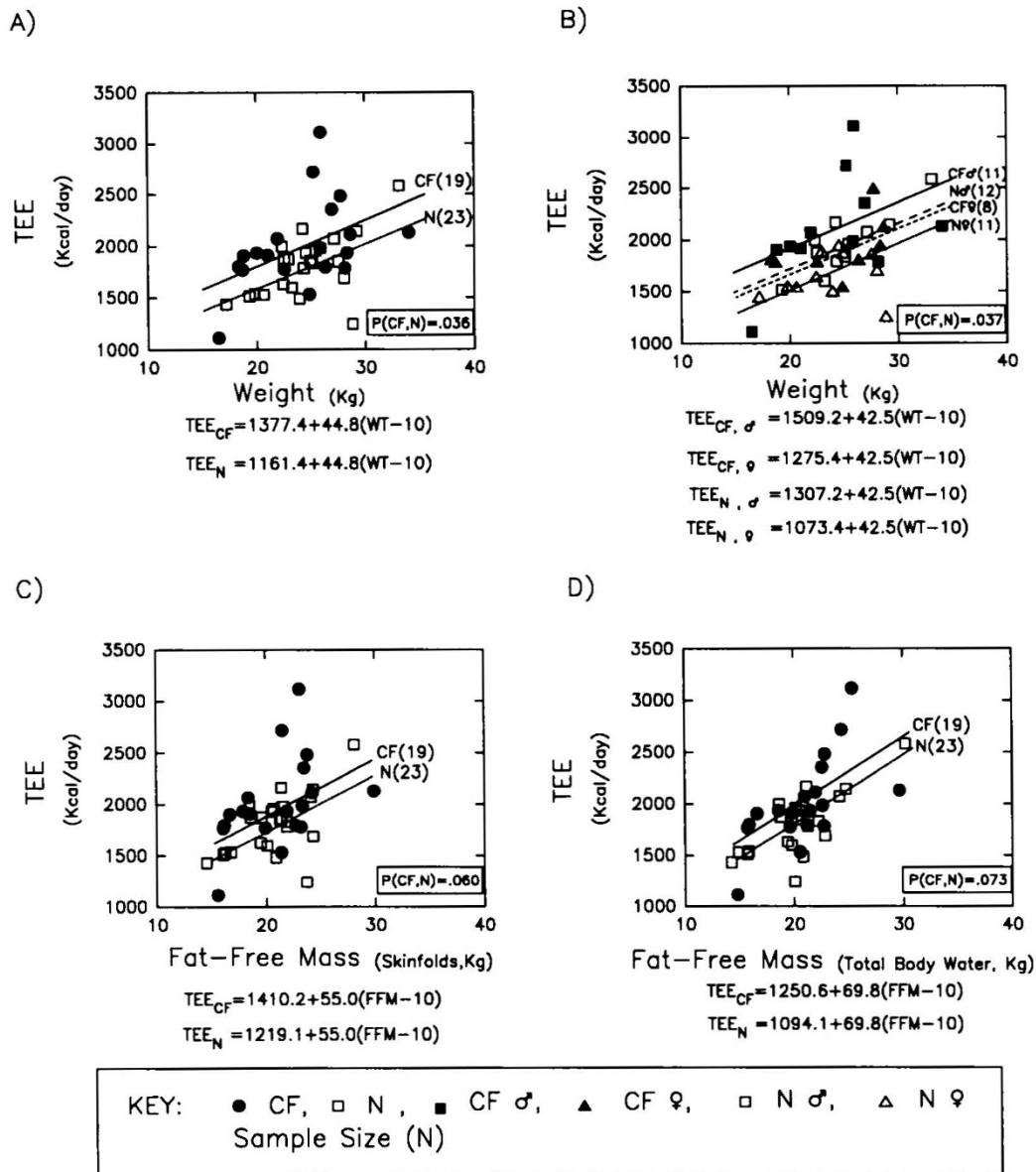


Fig. 3. Analysis of covariance of TEE by weight (A), weight and gender (B), FFM from two SF measurements (C), and FFM (D) from TBW in children with CF (CF) and control children (N).

($\Delta F508$ homozygous compared with all others) or of mild pulmonary dysfunction. Second, TEE was about 15–18% greater in children with CF compared with controls. The statistical significance of this increase was documented by sensitivity analysis methods, including estimation of missing TEE values. Third, the increase in CF TEE was attributed principally to the $\Delta F508$ homozygous subgroup and was not explained by pulmonary function, the season during which TEE was measured, or use of bronchodilator medications. Vaisman *et al.* (33) found that REE was increased by use of salbutamol in patients with CF. Physical activity, as reflected by the TEE/REE ratio, was increased in the $\Delta F508$ homozygous subgroup. It appears that in this CF subgroup with mild lung disease no compensation by decreased physical activity occurred, as described by Spicher *et al.* (10). However, the subjective activity score from parental observations showed no difference in physical activity between the CF and control groups or between the two CF subgroups and controls. It is generally noteworthy that statistical control for influential covariates is critical for detecting small differences between groups; however, caution is still required in interpreting the results from these models.

The CF REE values reported here are similar to those of

Buchdahl *et al.* (8), which were 7–9% above predicted using a regression equation generated from a comparable control group (Table 4). Their patients with CF were children and adolescents with highly variable lung function. Vaisman *et al.* (7) found REE in patients with CF to be 18% above predicted using the Harris-Benedict equations (34); however, these equations are inappropriate for prediction in their younger subjects. Furthermore, Vaisman's patients with CF varied with respect to age and lung disease. Fried *et al.* (11) described a curvilinear relationship with increasing REE and decreasing pulmonary function in male patients with CF and variable lung disease.

Our patients with CF represent a restricted range of mild to moderate lung disease where pulmonary function was not a major contributing factor to REE. The 6–9% increase in REE may be related to a number of factors, including an energy-requiring cellular defect; energy expenditure related to unmeasured, mild chronic pulmonary inflammation and infection; and an increase in work related to pulmonary disease not detected by these pulmonary function tests.

REE and genotype association was examined by Fried *et al.* (11), who found no relationship, and by O'Rawe *et al.* (12), who described an increased REE in the $\Delta F508$ homozygous subgroup.

Table 4. Studies of REE and TEE in patients with CF*

Author	Age in years (range)	n (M/F)	Pubertal status	Nutritional status	FEV ₁ (% predicted)	REE or TEE
Vaisman <i>et al.</i> (7)	9-36	71 (47/24)	Mixed	Mixed IBW 95 ± 14	61 ± 22 Severe-excellent	REE M: 120% pred F: 116% pred Harris-Benedict TEE: NA
Buchdahl <i>et al.</i> (8)	5-17	23 (12/11)	Mixed	Mixed Wt z score -0.7 Ht z score -0.4 Wt/Ht z score -0.5	83 (range, 40-120) Severe-excellent	REE 9.2% ↑ (wt, sex) 7.2% ↑ (FFM, sex) TEE: NA
Shepherd <i>et al.</i> (9)	0.7-2	9 (4/5)	Prepubertal	Underweight-wt percentile = 15th (range, 3-50)	NA	REE: NA DLW-TEE (8 d) ↑ 24% for age ↑ 27% for wt
Spicher <i>et al.</i> (10)	8-24	13 (7/6)	Mixed	Mixed IBW 81-97%	63 ± 5.5 SEM % Moderate-mild	REE 13% ↑ kcal/d 9% ↑ kcal/kg FFM/d HRM-TEE (2 d) No Δ kcal/d No Δ kcal/kg TEE/REE 1.56 CF 1.73 controls
Fried <i>et al.</i> (11) Group 1	7-39	32 (32/0)	Mixed	Good IBW >85%	76-116 Mild-excellent	REE % WHO kcal/d kcal/kg kcal/kg FFM TEE: NA
Group 2	7-39	61 (61/0)	Mixed	Good IBW >85%	35-114 Severe-excellent	REE 105-140% pred WHO TEE: NA
O'Rawe <i>et al.</i> (12)	7-15	78 (52-26)	Mixed	Mixed % BMI 91-102	48-102 Severe-excellent	REE % pred HBE TEE: NA
Tomezsko <i>et al.</i> (current study)	6-9	23 (11/12) REE 19 (11/8) TEE	Prepubertal	Good IBW 86-117%	61-117 Moderate-excellent	REE % WHO DLW-TEE (9 d) TEE kcal/kg TEE/REE
						$\frac{\Delta F508/\Delta F508}{104 \pm 5}$ 32 38 $\frac{\Delta F508/\text{other}}{105 \pm 9}$ 33 38 $\frac{\Delta F508/O}{109}$ O/O 104

* BMI, body mass index; IBW, ideal body weight; HRM, heart rate monitor; DLW, doubly labeled water; NA, not applicable; HBE, Harris-Benedict equations; WHO, World Health Organization equations.

The latter results may be an artifact because of the use of the Harris-Benedict equations for nonadult subjects or because of the effect of severe lung disease and malnutrition in their CF group.

The TEE results reported here are similar to those of Shepherd *et al.* (9), who found a 27% increase in TEE in clinically well infants and children with CF using the doubly labeled water method. Discussion of the interpretation of these data has centered mainly on the adequacy of the control group (35–38). Spicher *et al.* (10), using the 48-h heart rate monitor method for TEE, found no difference between CF and control groups despite higher REE for patients with CF. This lack of difference was attributed to reduced voluntary physical activity, *i.e.* compensation in the patients with CF.

The exclusion of eight TEE values as a result of unacceptably high variability in $r\text{CO}_2$ was probably due to contaminated samples or inaccurate analysis, not to disparate subject characteristics. One explanation for our 23% higher TEE in the ΔF508 homozygous subgroup is that voluntary physical activity was higher during the metabolic period. The four outlier patients may have exhibited extremes of activity, either typical or not, in their normal environment. Compensation by decreased physical activity as suggested by Spicher *et al.* (10) was not observed. TEE/REE ratios, which are influenced mainly by physical activity, support this explanation, although the activity scores from parental observations do not. The ΔF508 homozygous subgroup ratio (1.85) was close to reference values for normal children (1.8 to 1.9) (39); however, the ratios of the other subgroups were lower. In comparison, the TEE/REE ratios reported by Spicher *et al.* (10) were 1.56 for CF and 1.73 for control subjects. Another explanation for higher TEE is that all three groups had similar levels of physical activity but that the energy cost of physical activity was greater in the homozygous subgroup. Grunow *et al.* (40) reported that energy expenditure was significantly increased during activities of daily living in normally nourished female patients with CF and that this incremental increase in energy from the resting state to each activity level was comparable in CF and control groups. Their study did not assess subjects by genotype. Future studies of TEE, combined with more direct measures of physical activity, are needed.

Isotopic fractionation is an important issue in the clinical use of the doubly labeled water method and refers to the lower rate of vaporization of heavier isotopes compared with the lighter isotopes of the same element. It is assumed that breath water vapor and insensible transcutaneous water are fractionated and that sweat and urine are not (18). We assumed that isotopic fractionation was the same for patients with CF and control subjects. CF results in abnormal amounts of sodium and chloride in the sweat without affecting the insensible water losses from the cutaneous layer. CO_2 concentration was assumed to be 3.5% of expired air for the fractionation calculations. To verify this, we measured CO_2 concentration in four patients with CF (mean \pm SD, $2.5 \pm 0.5\%$) and five control subjects (mean \pm SD, $3.1 \pm 0.8\%$) in this study. Assuming a CO_2 value of 2.5% resulted in a 1.8% lower $r\text{CO}_2$ and 2.6% higher $r\text{CO}_2$ for maximal and minimal breath water loss, respectively. Because of these small $r\text{CO}_2$ differences, the original assumption of Schoeller *et al.* (18) (3.5% CO_2 and saturated breath water vapor) were used.

Clinical significance. Children with CF who were selected because of their mild lung disease, good nutritional status, and normal growth had a modest (6–9%) increase in REE. This increase could not be explained by genotype or degree of lung disease. TEE from normal daily activity was also increased in children with CF, but the increase of 15% was greater than the 6–9% accounted for by REE alone. When TEE was examined by genotype, the ΔF508 homozygous subgroup demonstrated a 22% greater increase compared with that of the control children. Unexpectedly, children with non- ΔF508 homozygous mutations had equivalent TEE to controls. Because of the mixed heterogeneous nature of this subgroup, definite conclusions could not be

drawn about its relationship to the ΔF508 homozygous subgroup or controls.

The increase in TEE in homozygous children may be related to increased voluntary physical activity as indicated by the TEE/REE ratio, although the parental activity score did not support this. Other causes include the possibility that the ΔF508 homozygous mutation caused a cellular or adaptive response resulting in an increased energy requirement for a given amount of physical activity. The clinical implication of this pattern of energy expenditure is that REE, either measured or estimated from equations, may not reflect the variability of physical activity and therefore may poorly predict the total daily energy needs of children with CF. Detailed assessment of current physical activity level for each individual with CF may be necessary to determine an accurate caloric goal for optimal growth or nutritional rehabilitation.

This information may provide a basis for the interpretation of previous studies (Table 4), explaining some of the conflicting findings expressed among studies because of varying subject populations. Recognition of the relationship between genotype and REE and TEE will aid our understanding of growth failure, malnutrition, and progression of pulmonary disease in children with CF.

Acknowledgments. The authors thank the children and their families and Sharon Sasanow, Betty Leifer, Dr. Edward Tomesko, Damary Vargas, Monica Darby, Joseph McDonough, Dr. Michael Bennett, Dr. Elsworth Buskirk, Dr. Penny Kris-Etherton, Dr. Dale Schoeller, and the General Clinical Research Center staff.

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