

A Six-Month Study of Growth and Energy Expenditure in Children with Cystic Fibrosis Taking a Pulmonary Inhalation Medication (rhDNase)

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Objective: To characterize the effects of recombinant human deoxyribonuclease (rhDNase) on growth velocity, body composition, resting energy expenditure (REE) and food intake in children with cystic fibrosis (CF).

Methods: A prospective, six-month pilot study was conducted in twenty-one subjects with CF (twelve male, nine female, ages 11.5 ± 3.1 years) measured at baseline, two and six months post-baseline. Repeated measures ANOVA was used to examine the change in variables across time.

Results: The majority (75%) of subjects had minimal lung disease at baseline (FEV_1 : 80%–119% predicted). As expected for growing children, weight and height gains (1.6 kg and 2.5 cm) were observed between baseline and six months ($p=0.0001$). No change was observed in weight z-scores from six months prior to initiation of rhDNase therapy to six months post, though a significant decline ($p=0.049$) in Ht z-score was observed over this twelve-month period. Triceps skinfolds and mid-arm muscle circumference increased from baseline to six months ($p<0.01$); respective z-scores remained stable. Energy intake remained constant during the period it was studied from baseline to two months of therapy: $120\% \pm 27\%$ RDA. REE, though slightly elevated compared to healthy children (baseline $106\% \pm 8\%$ predicted), remained stable throughout the study and at a level which may be expected for children with minimal lung disease. A trend ($p=0.057$) towards a decrease in the number of subjects requiring hospitalization for pulmonary exacerbations during the trial period was observed.

Conclusions: In summary, these pilot data from younger children with milder CF-related lung disease do not confirm anecdotal reports of improved rate of weight gain, caloric intake or decreases in the elevated REE. Future research might focus on documentation of the possible nutritional effects of rhDNase in clinical trials of children with more severe lung disease.

INTRODUCTION

Cystic fibrosis (CF), the most common life-threatening autosomal recessive disorder in Caucasians [1], is caused by single gene mutations on the long arm of chromosome 7. The

gene encodes for the CF transmembrane conductance regulator, a cyclic-AMP regulated chloride channel [2]. CF affects the lungs, as well as the pancreas, hepatobiliary system and gastrointestinal tract. The course of the disease is characterized by

Abbreviations: CF=cystic fibrosis, FEV_1 =forced expiratory volume in 1 second, $FEF_{25\%-75\%}$ =forced expiratory flow at 25%–75% of vital capacity, FFM=fat-free mass, FVC=forced vital capacity, GCRC=General Clinical Research Center, rhDNase=recombinant human deoxyribonuclease, commercially known as Pulmozyme®, RDA=Recommended Dietary Allowance, REE=resting energy expenditure, WHO=World Health Organization.

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multiple hospitalizations and growth failure [3]. Despite recent improvements in clinical care, pulmonary disease remains the leading cause for hospitalization and the major cause of death in patients with CF [4].

Among children with CF, progression of pulmonary disease and declining nutritional status are often the dominant clinical concerns. Although there is no evidence that the CF gene defect directly codes for short stature, poor weight gain or abnormal body composition, children with CF typically have growth failure. The 1997 CF Foundation Patient Registry [4] reports that 19% of patients were less than the 5th percentile for height and 24% less than the 5th percentile for weight. Better nutritional status is associated with longer survival and with improvement or stabilization of pulmonary function in malnourished patients [5].

Children with CF have increased resting energy expenditure (REE), likely contributing to the commonly seen growth failure [6–11] when not balanced by increased energy intake and/or absorption. Non-hospitalized patients with CF show elevations in REE ranging from 4% to 25% higher than control subjects or predicted values derived from healthy children. For those with relatively good nutritional status and mild pulmonary disease, REE is only modestly elevated [6].

A recent addition to the care of patients with CF is the use of the medication dornase alfa (commercially known as Pulmozyme®; Genentech, San Francisco, CA). Dornase alfa is a highly purified, FDA approved, inhalation solution of recombinant human deoxyribonuclease (rhDNase I). This enzyme has the specific function of cleaving extracellular DNA and reduces sputum viscosity *in vitro* [12]. By 1996, at the time of this study and after two years of clinical availability of rhDNase, 45% of all patients in the US with CF had received the drug as part of clinical care [4]. The efficacy and safety of inhaled rhDNase was documented in clinical trials [13,14] and in review articles [15]. In one large, longitudinal, randomized, placebo-controlled trial, rhDNase was evaluated in clinically stable subjects older than five years of age with CF, with baseline FEV₁ 60±27 (% of predicted) reflecting moderately severe lung disease [14]. Subjects were treated for six months with either placebo or one of two dosing regimens of rhDNase, 2.5 mg, either once or twice daily. Both treatment groups had significant reductions in the number of hospital days and respiratory tract infections requiring parenteral antibiotics and showed improvement in the predicted FEV₁ compared to the control group. Improved appetite, sense of well-being and weight gain were anecdotally reported in some of the subjects who participated. By improving pulmonary function, rhDNase may also affect REE. Anim and Dozor [16] found that in five adult patients with CF whose severe lung function (FEV₁ range at baseline: 30–46% predicted) improved in response to treatment with rhDNase for two weeks, a proportional decline in REE also occurred.

These reports stimulated initiation of this preliminary study to characterize the effects of rhDNase on nutritional status in children with mild CF related lung disease. The aim was to

evaluate growth velocity, body composition, REE, dietary intake and pulmonary function prospectively over a six month period (the same time interval as the large clinical trial) in subjects prescribed rhDNase as part of their usual clinical care. The hypotheses were that rhDNase therapy would be associated with an overall improvement in nutritional status: an increase in linear growth, weight gain velocity, and dietary intake after six months of treatment, and that these improvements would be mediated by a decrease in REE.

METHODS

Subjects

Over a twelve month period, children older than six years of age with CF and pancreatic insufficiency who were prescribed rhDNase for clinical care were recruited from the CF Center at the Children's Hospital of Philadelphia to participate in a six-month prospective study. The diagnosis of CF was based on clinical symptoms and duplicate quantitative pilocarpine iontophoresis sweat tests with Na and Cl values ≥60 mEq/L. At initial diagnosis of CF, pancreatic insufficiency was determined by a stool trypsin value of <80 µg/g and/or 72 hour fecal fat analysis with <93% absorption. Subjects with gastrostomy feeding tubes, *Burkholderia cepacia* or other chronic illnesses that may affect growth and development (e.g., insulin dependent diabetes mellitus, Crohn's disease) or who were hospitalized for any reason within two weeks of the baseline measurement were excluded. The protocol was approved by the Institutional Review Board at the Children's Hospital of Philadelphia. Informed written consent was obtained from the parents of each subject, and assent was obtained from the child. The cost of rhDNase therapy was paid by the families and/or insurance companies. Genotype was not determined as part of this study and was available for only seven subjects. Enrollment in this study did not influence any other aspect of usual clinical care.

Study Protocol and Clinical Care

Subjects were evaluated during a 20-hour overnight admission to the General Clinical Research Center (GCRC) at three time points: baseline (before initiating rhDNase therapy) and after two and six months of therapy. All subjects were treated according to the standard CF care at our facility. RhDNase, 2.5 mL/day (as a 1.0 mg rhDNase/mL solution of 150 mM NaCl, 1.5 mM Ca Cl: pH 6.0), was inhaled daily using a compressor (Debilviss Pulmoaid) and nebulizer (Hudson T-2 Updraft or Marquest Acorn-2) for a period of six months. Subjects and families who agreed to participate in this study were historically compliant with previous medication protocols, and because rhDNase was taken during the usual morning regimen in most cases, compliance generally was optimal. Nine of the 21 subjects were previously exposed to rhDNase therapy in an

independent two-week research protocol designed to compare the effect of differences in aerosol particle size, delivered by two different nebulizers, on pulmonary function [17]. The particle size protocol was completed three to ten months prior to the initiation of the current protocol, and subjects enrolled in the current protocol were not treated with rhDNase during the interim.

All of the variables described below were measured at each time point with the exception of bone age, which was determined at baseline only, and chest x-ray, which was taken at baseline and at six months. Compliance with rhDNase therapy was evaluated by home calendar reporting and confirmed by interviews of subjects and parents at each visit. The number of hospital day stays was collected by chart review. Side effects were noted at the time of study visit, by interview.

Anthropometry, Skeletal Age and Pubertal Status

Body weight, standing height, skinfolds at four sites (triceps, biceps, subscapular, suprailliac) and mid-arm circumference were measured by a single trained anthropometrist using standardized techniques [18,19] at baseline, two and six months. All measurements were taken in triplicate and the average was used for analysis. Weight and height values for six months prior to baseline measurements were obtained from the CF Clinic charts for which similar anthropometry equipment and protocol are used. Short term growth was measured using a knee-height measuring device which measures growth of the lower leg (mm) as described by Cronk *et al.* [20]. Five measurements were taken and the mean and standard deviation computed. If the standard deviation was greater than 0.5 mm, the five measurements were repeated and the new mean was used in the analysis. Weight and height values were compared with National Center for Health Statistics reference standards [21], and z-scores were computed (Centers for Disease Control Anthropometric Software Program, Version 3.1, 1988, Division of Nutrition, Centers for Disease Control, Atlanta, GA). Weight for height z-scores were calculated according to Cole [22]. Triceps z-score was computed using reference data from Frisancho [23]. Mid-arm muscle circumference and mid-arm muscle area were computed from mid-arm circumference and triceps skinfold data using standard equations [20] and z-scores computed using reference data [23]. Knee height velocity for those subjects between the ages of six to ten years was compared to reference data from 103 subjects who did not have CF of the same age group using the same measuring device [24]. Weight and height velocity were compared to data from the Fels Institute Longitudinal Growth Study [25] and z-scores were determined based on subjects' age and gender. Fat-free mass (FFM), fat mass and percentage body fat were determined using two methods (two or four skinfold sites) from age- and gender-specific prediction equations [26–28].

Skeletal maturation was determined at baseline by radiological examination of the left hand and wrist. The Tanner-Whitehouse II method [29] was used by a single observer to assign a maturity score from 20 bones and bone age was determined. Pubertal development was assessed by self-examination [30] with parental assistance using a self-examination pictorial questionnaire and scored from one to five based on pubic hair and breast or genital developmental according to the method of Tanner [31]. Tanner stages are presented as a combined score in this report.

Dietary Intake and Resting Energy Expenditure (REE)

Food intake was determined by three-day weighed food records at baseline and two-month time points [32]. Intake data were analyzed using a nutrient database program (Food Processor Plus, Version 5.03, ESHA Research, Salem, OR). Dietary intake data were averaged over the three days of records and represented as a percentage of the Recommended Dietary Allowance (RDA) [33], as well as a percentage of the CF specific dietary recommendations [34].

REE was measured by open circuit indirect calorimetry between 0700 and 0900 after a 12- to 14-hour overnight fast while subjects were admitted to the GCRC and prior to administration of morning inhalation treatments or chest physiotherapy. A computerized metabolic cart (SensorMedics 2900 Z, SensorMedics, Yorba Linda, CA) was used to measure REE for 60 minutes in a quiet thermoneutral room. Subjects rested in a supine position while under a large, clear, ventilated hood. Sampled expiratory gases were analyzed every second, and one minute averages were recorded. The first ten minutes were devoted to environmental adjustment by the child and were eliminated from calculations. Data points derived during documented periods of significant movement or coughing that were associated with changes in REE values were eliminated and the remaining data averaged for analysis. The REE was calculated from the oxygen consumption and carbon dioxide production by equations described by Weir [35]. The coefficient of variation for REE measurement was $5 \pm 3\%$ based on REE measurements recorded under similar conditions in seven children with CF (nine to 15 years of age) measured on three consecutive days during a clinically stable period, as previously described [11]. REE data were expressed as kcal/d, kcal/kg/d and as percent of the predicted values from the World Health Organization (WHO) equations [36] in order to compare individuals of different age, gender and weight.

Clinical Status

Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and forced expiratory flow at 25% to 75% of vital capacity ($FEF_{25\%-75\%}$) were measured using standard methods for spirometry [37], and expressed as a percentage of the reference values using Knudsen equations [38]. Chest x-ray

score was determined by averaging the scores determined by two pulmonary physicians blinded to subject identity or clinical status, who independently evaluated the films using the Brasfield system [39].

Statistical Analysis

Repeated measures analysis of variance was used to examine change in variables across time. Longitudinal mixed effects analysis (Stata, Version 5.0; College Station, TX) was used to estimate the effects (age, gender, FFM) on REE both within and between subjects over time. This type of regression analysis adjusts for the linear effect of the covariate (FFM) on REE at each measurement [40]. Correlations were determined by Pearson's correlation coefficient. Comparisons between body composition methods were made using Student's *t* test and correlation coefficients. Differences were considered statistically significant at a probability level of $p < 0.05$, and results are presented as mean \pm SD.

RESULTS

At the initiation of this study approximately 25% of patients seen at the Children's Hospital of Philadelphia CF center, who were greater than six years of age, were already taking rhDNase on a regular basis and were therefore excluded from this investigation. Twenty-six subjects (16 male, ten females) with CF were enrolled in the study, and 21 subjects (13 male, eight female), completed the six month protocol. Two subjects (one male, one female) withdrew after the baseline measurement due to financial considerations related to rhDNase. Three others withdrew before the six month time point. These included one male who stopped taking rhDNase after the two month time point, one female who withdrew because of psycho-social difficulties and one male who withdrew because his scratchy throat inhibited him from singing in a school play. No differences were observed in the disease activity, hospital admissions or pulmonary function between the five subjects who withdrew and those who completed the study. The mean age of the subjects at baseline measurement was 11.5 ± 2.9 years (range 7 to 16), the mean bone age was 11.3 ± 3.6 (range 6 to 18) and combined Tanner score for sexual development was 2.4 ± 1.4 (range 1 to 5). Side effects of rhDNase therapy that were reported by the subjects included coarse/hoarse voice ($n=3$) and runny nose ($n=1$).

All subjects were maintained under standard medical care for CF throughout the six-month trial. The subjects were on combination nebulizer therapy (albuterol, cromolyn sodium acetylcysteine), oral antibiotics (co-trimoxazole combined with cefaclor or cephalexin) and/or histamine-2 receptor blockers (mainly ranitidine hydrochloride). At the initiation to the study the pancreatic enzyme dose was on average 4058 ± 3155 U

lipase/kg/meal. Individual enzyme dosages decreased on average by 9% during the six-month interval. All subjects were taking a multivitamin supplement twice daily, and vitamins E and K as recommended [34]. One subject began oral prednisone one month before the end of the project, and two subjects used triamcinolone during the study (one from baseline, the other from the two-month time point). Three subjects were on lactulose to prevent constipation or small bowel obstruction. Two subjects were diagnosed with attention deficit hyperactivity disorder prior to the initiation of the study and were taking methylphenidate hydrochloride throughout the six month period. For these two subjects, methylphenidate hydrochloride was continued on the same time interval and dose as prior to initiation of the REE measurement for consistency. Two other subjects were taking ursodeoxycholic acid secondary to sludge in the gallbladder and hyperechogenicity of the liver on ultrasound. Neither of the two subjects had significant elevation of liver enzymes or evidence of hepatic synthetic dysfunction.

Growth and Body Composition

Indices of growth and body composition are presented in Table 1. Weight and height gains of 1.73 ± 2.54 kg (range: -1.24 to 7.14) and 2.77 ± 20.1 cm (range: -0.48 to 5.86) were observed between six months prior to initiation of the study and the baseline measurements ($p=0.0001$). While subjects were taking rhDNase, gains of 1.62 ± 1.48 kg (range: 0.00 to 5.81) and 2.48 ± 1.60 cm (range: -0.43 to 5.37) were observed between the baseline and six month evaluation ($p=0.0001$). Seven pre- and peri-pubertal subjects had weight gains of less than 0.5 kg in the six months prior to rhDNase therapy (two female, five male) compared to three pre and peri-pubertal subjects with similar poor weight gain while on rhDNase (two female, one male).

The observed gain in weight during the rhDNase trial was the result of increases in FFM and fat mass, calculated from equations using skinfold measurements taken at both two or four sites (Table 1). FFM and fat mass by the two methods were highly correlated at all time points (e.g., FFM at Baseline: two sites vs. four sites: $r^2=0.998$). Percentage total body fat did not change during the study. Mean triceps skinfold thickness improved progressively from baseline to six months (Table 1: $p=0.002$). When triceps z-score was calculated from reference data for Caucasian children of similar age, there was no significant improvement across time ($p=0.08$).

Knee height, an accurate indicator of linear growth over short time intervals, increased 2.91 ± 1.88 mm from baseline to two months and 9.70 ± 3.82 mm over the six month time point in all subjects (Table 1). During the same six-month interval, knee height increased 10.31 ± 1.61 mm in those children aged six to ten, for whom reference data are available [24]. In these eight children below the age of ten, knee height velocity for the two month interval was 3.99 ± 1.67 mm (range: 0.88 to 6.07),

Table 1. Baseline, Two and Six Months Measurements (Mean±SD) of Growth and Body Composition in 21 Children with CF Prescribed rhDNase

	Time (Months)			p Value ¹
	Baseline	Two	Six	
Growth measures				
Weight, kg	36.5 ± 15.5 ^a	36.9 ± 15.9 ^a	38.1 ± 16.1 ^b	0.0001
Height, cm	142.4 ± 18.6 ^a	143.3 ± 18.6 ^b	144.8 ± 18.7 ^c	0.0001
Knee height, mm ²	243 ± 65 ^a	246 ± 64 ^b	252 ± 64 ^c	0.0001
Subscapular, mm	5.8 ± 1.8	5.8 ± 2.0	5.9 ± 1.8	NS
Tricep, mm	7.9 ± 2.4 ^a	8.2 ± 2.6 ^b	8.6 ± 2.5 ^c	0.002
Mid-arm circumference, mm	208.9 ± 38.9 ^a	211.1 ± 41.6 ^b	214.6 ± 43.5 ^c	0.0003
Mid-arm muscle circumference, mm	183.9 ± 38.0 ^a	184.9 ± 41.2 ^{ab}	187.6 ± 43.3 ^b	0.022
Mid-arm muscle area, cm ²	28.0 ± 12.6 ^a	28.5 ± 14.1 ^{ab}	29.4 ± 15.2 ^b	0.023
Body composition				
Two Skinfolds³				
Fat free mass, kg	32.1 ± 13.9 ^a	32.3 ± 14.3 ^a	33.2 ± 14.5 ^b	0.0001
% body fat	12.0 ± 3.8	12.4 ± 4.0	12.8 ± 3.8	NS
Fat, kg	4.4 ± 2.4 ^a	4.6 ± 2.5 ^a	4.9 ± 2.5 ^b	0.0017
Four Skinfolds⁴				
Fat free mass, kg	31.1 ± 12.7 ^a	31.3 ± 13.1 ^a	32.3 ± 13.4 ^b	0.0001
% body fat	14.1 ± 4.7	14.3 ± 5.3	14.5 ± 4.4	NS
Fat, kg	5.5 ± 3.4 ^a	5.7 ± 3.4 ^a	5.9 ± 3.3 ^b	0.04

¹ Superscripts that differ from one another denote statistical differences between timepoints.

² n=18, for knee height measurement.

³ Equations developed by Slaughter *et al.* [28] based on two skinfold sites.

⁴ Equations developed by Brook [26] for subjects ≤11 years or Durnin and Rahaman [27] for subjects >11 years, based on four skinfold sites.

compared to 3.77±1.43 mm for the reference group during the same interval, indicating a growth rate between the 50th and 75th percentile for age and gender.

Z-scores and NCHS percentiles for growth and body composition are presented in Table 2. Mean z-scores for weight (-0.61±0.92) and height (-0.61±1.08) were below reference values at six months prior to initiation of the study as well as at the baseline measurement (weight z-score -0.67±0.92 and height z-score: -0.67±1.04). Weight z-score remained stable during rhDNase therapy, though height z-score declined significantly by the end of six months of therapy (p=0.049;

-0.72±1.10). At baseline measurement, approximately 10% of the subjects were below the 5th percentile for weight, while 24% of the subjects were below the 5th percentile for height. Growth percentiles did not change significantly during the six months prior to initiation of the study nor during the six months of the rhDNase trial.

Using published growth velocity data from normal healthy growing US children [25], weight and height velocity z-scores were calculated for the six-month study interval. Weight velocity z-score was -0.37±0.84 (range: -1.61 to 1.63) and height velocity z-score was 0.25±1.56 (range: -2.31 to 4.17).

Table 2. Pre-study, Baseline, Two and Six Months Calculated z-Scores (Mean±SD) of Growth and Body Composition in 21 Children with CF Prescribed rhDNase

	Time (Months)			
	Pre-Study ¹	Baseline	Two	Six
Weight z-score ²	-0.61 ± 0.92	-0.67 ± 0.86	-0.72 ± 0.93	-0.72 ± 0.91
Weight for age percentile	31.7 ± 27.9	29.7 ± 26.3	29.2 ± 27.9	28.9 ± 27.2
Height z-score ^{2,3}	-0.61 ± 1.08 ^a	-0.67 ± 1.04 ^{ab}	-0.67 ± 1.06 ^{ab}	-0.72 ± 1.10 ^b
Height for age percentile	34.2 ± 31.3	31.1 ± 29.9	31.7 ± 29.8	31.0 ± 30.2
Weight for Height z-score	-0.28 ± 0.57	-0.28 ± 0.59	-0.36 ± 0.67	-0.32 ± 0.57
Triceps z-score ²		-0.67 ± 0.09	-0.58 ± 0.12	-0.56 ± 0.10
Upper arm circumference z-score		-0.70 ± 0.14	-0.70 ± 0.16	-0.67 ± 0.16
Upper arm muscle circumference z-score		-0.42 ± 0.17	-0.47 ± 0.21	-0.49 ± 0.21

¹ Pre-study weight and height z-scores were calculated from data collected six months prior to entry into the study.

² Weight and height z-scores were calculated using Centers for Disease Control Anthropometric Software Program, Version 3.1, 1988, Division of Nutrition, Centers for Disease Control, Atlanta, GA; weight for height z-scores calculated from reference data found in Cole *et al.* [22] and the body composition z-scores were calculated using reference data from Frischancho [23].

³ Superscripts that differ from one another denote statistical differences between timepoints. p-value for height z-score=0.049.

Pulmonary Function and Hospitalizations

The majority of subjects (16 of 21) had pulmonary function within the normal range at baseline (FEV₁: 80% to 119% predicted); the remaining five subjects had mild lung disease (FEV₁: 67% to 79% predicted). No significant changes were observed across time in the pulmonary function tests (FEV₁, FEF_{25%-75%}, FVC (Table 3). Statistical analysis was also performed separately on the five subjects with mild lung disease (baseline FEV₁ <80%). Though FEV₁ appeared to increase from a baseline value of 76% ± 5% to 88% ± 21% at the six month time interval, no statistical differences were observed (p=0.31).

The number of subjects requiring hospitalization due to pulmonary exacerbation dropped from six to two, and approached statistical significance (p=0.057), when the six-month period prior to initiation of rhDNase was compared with the six months of therapy. Comparing the two six-month periods, the number of total hospital days for all subjects dropped from 68 to 14 (p=0.055). The six subjects who were admitted to the hospital during the six months prior to the rhDNase trial had FEV₁ averaging 91% ± 15% predicted, which was not different from that of the entire study population.

Resting Energy Expenditure

Using the mixed effects regression model to control for the effects of age, gender, and FFM (from two-site skinfold method) on REE (kcal/d), no statistically significant differences were observed across time. In addition, there was no statistically significant change in REE when expressed in absolute terms (kcal/day) or when expressed per kg of body weight (kcal/kg/d) (Table 3). REE, expressed as a percentage of the WHO prediction equations, which adjusts for age, weight and gender, also did not differ between time points. Percent WHO

Table 3. Baseline, Two and Six Months Pulmonary Function and Resting Energy Expenditure (Mean ± SD) in 21 Children with CF Prescribed rhDNase

	Time (Months)		
	Baseline	Two	Six
Pulmonary function			
FVC, n=18	103 ± 12	102 ± 16	103 ± 13
FEV ₁ , n=18	94 ± 14	93 ± 16	92 ± 14
FEF ₂₅₋₇₅	81 ± 33	81 ± 29	74 ± 31
Chest X-Ray Score ¹	19.5 ± 1.7	—	19.4 ± 1.4
Resting energy Expenditure			
Kcal/day	1334 ± 345	1364 ± 403	1387 ± 441
Kcal/kg/day	39.1 ± 7.6	39.4 ± 7.8	38.5 ± 7.3
% WHO ²	106 ± 8	107 ± 12	107 ± 12

¹ Based on the 25 point scale developed by Brasfield *et al.* [39].

² Determined according to World Health Organization Equations [36].

value for individual subjects varied from 93% to 122% at baseline.

Dietary Intake

Dietary intake are presented in Table 4 as the average of three-day weighed food records. Nineteen subjects completed the records at both the baseline and two month time period. There were no statistically significant differences with respect to age, gender or FEV₁, between those subjects who completed the records and those who did not. Dietary intake did not change between baseline and two months and averaged 120% ± 27% RDA. Subjects consumed more than the amount recommended for healthy children and near the goals for CF children [34] based on their age, gender, weight, activity level, pulmonary function and fat absorption. Macronutrient composition, the percentages of calories from fat, protein and carbohydrate of the diet, remained stable across this two month period.

In summary, no statistically significant correlations were observed between absolute values of REE, energy intake, and/or pulmonary function, nor were correlations observed between the percentage change in REE, energy intake, pulmonary function and/or anthropometric indices.

DISCUSSION

This is the first study to prospectively investigate the effects of rhDNase therapy on growth, body composition, energy expenditure and dietary intake in young subjects with mild CF-related lung disease over a six-month time interval. We observed an increase in the absolute measures of growth (weight, height, triceps skinfold thickness, arm circumferences, arm muscle area) during the six-month interval, which is expected of healthy growing children. Overall, we saw no clinically significant changes in nutritional status in these children taking rhDNase for six months.

Table 4. Baseline and Two Months Dietary Intake (Mean ± SD) in Children with Cystic Fibrosis Prescribed rhDNase (n=19)

	Time	
	Baseline	Two Months
Kcal/day	2461 ± 447	2424 ± 714
Kcal/kg/day	75 ± 24	70 ± 20
Kcal/d; % RDA ¹	122 ± 27	118 ± 27
Kcal/d; % CF Goals ²	105 ± 22	100 ± 20
% Total Kcal, Fat	33 ± 5	35 ± 5
% Total Kcal, Protein	14 ± 2	14 ± 2
% Total Kcal, Carbohydrate	53 ± 6	51 ± 6

¹ RDA: US Recommended dietary allowance for healthy children [33].

² CF Recommendations based on age, weight, FEV₁, fat absorption (coefficient of fat absorption arbitrarily set at 1.1) and activity level [34].

This sample of children and adolescents with CF had lower weight, height, and triceps z-scores compared to reference data from healthy children [21,23], indicating poorer growth and nutritional status in the sample overall at baseline. However, this sample was similar with respect to mean weight and height percentiles relative to the national CF patient registry [4]. At baseline, twenty-four percent of these subjects were below the 5th percentile for height, although, only 10% were below the 5th percentile for weight, compared to 24% for the national registry. This suggests that the number of children with extreme underweight in the present sample was lower than the national average. The lower prevalence of subjects under the 5th percentile for weight may be influenced by the majority of subjects with pulmonary function in the normal range.

We observed a statistically significant increase in weight and height over the six-month rhDNase therapy period. Using reference data from the growth velocity charts for US children, weight velocity z-score was slightly negative while height velocity z-score, slightly positive. There was a large range in the individual pattern of growth for both weight and height. However, the mean rate of growth in this sample of children with CF and mild pulmonary disease was similar (e.g., within ± 0.5 z score) to healthy growing children. Our results from knee height velocity also support the conclusion that these children are growing similarly to healthy non-CF children. Although there are limited reference data available for knee height velocity, for those subjects for whom reference data are available, results showed growth in the 50th to 75th percentile range for normal non-CF children. Recent analysis of the 1993 National CF patient registry concurs with this finding [41]. Lai *et al.* found that the occurrence of malnutrition in CF patients (defined as weight and height for age percentiles < 5 th) was at its lowest prior to the onset of adolescence.

A statistically significant increase in triceps skinfold thickness was documented, indicating improved energy stores at this subcutaneous site, though no significant improvement in triceps z-score was noted. The inability to observe statistical differences in z scores for body composition values across time in this study may have been complicated by two factors. Firstly, almost half of our subjects had birthdays during the six-month interval of the drug therapy trial. Therefore, their growth measurements were compared to two different age intervals, which may or may not have been appropriate, depending on how closely their ages corresponded with the reference ages. Secondly, the majority of the subjects were in the midst of their pubertal growth spurt; the average Tanner score for sexual maturation was 2.4 ± 1.4 . Measuring small samples of children during peripubertal development is always a confounding factor in growth studies. However, this age group was assessed purposefully, because this is often when the first decline in pulmonary function and nutritional status is noticed, and a six-month time interval was chosen to enhance compliance to the intervention.

In an effort to further evaluate the changes in growth,

nutritional status and disease progression, we compared the current findings with a three-year, prospectively study of growth and energy expenditure in 25 pre-pubescent children with CF compared to 26 control children [11]. Though the sample was slightly younger (five to ten years) at the beginning of the study compared to the present group, the two groups of children with CF were similar with respect to gender (57% M vs. 50% M), baseline weight and height z-scores (between -0.5 and -0.6 and pulmonary function (FEV_1 : $88\% \pm 17\%$ vs. $94\% \pm 14\%$). The girls with CF in the previous study experienced a significant decline in height z-score of -0.11 units/year, and the boys a decline in weight z-score of -0.09 units/year [10]. An overall decline in triceps z-score of -0.15 units/year was also observed. Due to differences in study design and sample characteristics, it would be inappropriate to statistically compare the two groups of subjects. The current group of children with CF maintained weight and triceps z-scores over the six-month time interval on rhDNase therapy; this is in contrast to the results expected from the previous three-year study of children cared for at the same CF Center.

No statistically significant changes were noted in the pulmonary function tests. Previous clinical trials have found significant improvement in pulmonary function as well as a decrease in the incidence of pulmonary exacerbations in those subjects taking rhDNase. However, all the previously reported studies were conducted on subjects with more advanced lung disease. The analysis of the pulmonary function results were repeated in the current study focusing on the small subgroup ($n=5$) of subjects with $FEV_1 < 80\%$ predicted, though no statistical differences were observed.

For these subjects, with only mild pulmonary disease, REE was found to be slightly elevated (approximately 6%) compared to the predicted values based on age and gender specific equations. REE remained stable across the three time points after accounting for the effects of age, gender and FFM by multivariate analysis. Elevated REE has been noted in previous cross-sectional studies of children and adolescents with variable levels of CF pulmonary disease [6–11]. In a report of children with CF with relatively good nutritional status and mild pulmonary disease, REE was similarly increased by 6% to 9% [6].

In our previous longitudinal study of children with CF, we observed a statistically significant increase in REE across time without a significant decrease in pulmonary function [11]. The increase was more pronounced in the girls with CF, for whom REE increased at a rate of 3.3% predicted per year. From these data, REE is an earlier indicator of changes in clinical CF status than pulmonary function because REE incremental alterations were observed before the decreases in lung function. In the present study, we observed no differences in pulmonary function and no change in REE. Thus, the lack of decline in REE observed in the current subjects may represent a positive clinical effect of rhDNase on energy expenditure.

Anecdotal reports from parents of the current subjects consistently suggested appetite had improved with rhDNase therapy, but no statistical changes were observed. Dietary intake was similar to that in many previously published reports both in total caloric intake and percentage of the RDA [6,32]. Even in cooperative subjects, accurate energy intake data are known to be difficult to obtain [42,43], and accurately assessing dietary intake in future studies may require the use of energy balance methods such as doubly labeled water [44].

CONCLUSION

These pilot data from younger children with mild CF-related lung disease do not confirm the anecdotal reports of improved rate of weight gain, caloric intake or decreases in the elevated REE. Future research might focus on documentation of the possible nutritional effects of rhDNase on children with more severe lung disease.

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