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Effects of Tolbutamide on Growth and Body Composition of Nondiabetic Children with Cystic Fibrosis

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ABSTRACT. Previously, we reported that nondiabetic children with cystic fibrosis show a blunted insulin response to a meal stimulus. In the study presented here, using tolbutamide, we determined the effects of augmented insulin secretion/action on height and lean body mass of children with cystic fibrosis. Twelve subjects (mean ± SEM age, 11.0 ± 0.5 y) were studied for three 4-mo periods: *1*) pretreatment, *2*) treatment, consisting of 750 mg/d of tolbutamide, and *3*) posttreatment. Before the pretreatment period, insulin response to a meal stimulus was evaluated in relation to three doses of tolbutamide: 0, 250, and 500 mg. Growth was monitored during each period, and incremental changes in lean body mass were calculated from height data. To validate the change in lean body mass based on height measurements, we determined lean body mass in seven subjects during the treatment period by using a criterion method ($H_2{}^{18}O$). Growth velocity (cm/4 mo) significantly increased ($p < 0.05$) during the treatment (2.58 ± 0.31) compared with the pretreatment period (0.88 ± 0.20). The increase in lean body mass calculated from height was greater during the treatment (1.61 ± 0.29 kg/4 mo) than during the pretreatment period (0.44 ± 0.18 kg/4 mo) ($p < 0.05$). There was also a significant increase ($p < 0.05$) in lean body mass during the treatment as measured with $H_2{}^{18}O$ (1.91 ± 0.65 kg/4 mo). Acute administration of either 250 or 500 mg of tolbutamide reduced ($p < 0.05$) the area under the glucose concentration curve in response to a meal compared with the control condition of no tolbutamide. However, there was no significant effect of tolbutamide on plasma insulin levels. We conclude that short-term tolbutamide therapy more than doubled linear growth and increased lean body mass accretion 4-fold in slowly growing children with cystic fibrosis, possibly by improving the tissue response to insulin. (*Pediatr Res* 30: 309–314, 1991)

Abbreviations

CF, cystic fibrosis
$H_2{}^{18}O$, isotopic form of water in which oxygen has an atomic weight of 18
LBM, lean body mass
TBW, total body water
CSC, Clinical Studies Center
ANOVA, analysis of variance
AUC, area under the curve
Rx, treatment
Pre-Rx, pretreatment control
Post-Rx, posttreatment control

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Malnutrition in children with CF is associated with poor pulmonary function, poor immune function, and poor growth (1-4). The cause of the malnutrition in CF may be a consequence not only of malabsorption but also of inadequate intake and increased energy expenditure (5–7). Aggressive nutritional therapy in patients with CF has contributed to improvements in pulmonary function, immune response, growth, the overall clinical course, and survival (1–4, 8, 9). However, in practice, high energy intakes may be difficult to achieve in some patients.

Functional insulin deficiency is a secondary complication of CF. It has been estimated that 40% of CF patients show glucose intolerance (10, 11) and low insulin levels in response to oral glucose tolerance tests (10, 12). Similarly, glucose intolerance and insulin deficiency have been observed during meal stimulation tests (13) and during i.v. glucose tolerance tests (14). Insulin deficiency is associated with significant abnormalities in the secretion of other pancreatic hormones including gastrointestinal peptide and pancreatic polypeptide (13, 14); however, we have demonstrated that, with the ingestion of a high-calorie meal, insulin and gastrointestinal peptide secretion can be increased (13). A nutritionally induced improvement in insulin secretion therefore could contribute to the observed improved growth and nutritional status directly by enhancing glucose utilization or via indirect effects on protein and amino acid metabolism (15–18).

The purpose of this study was to examine the effects of a 4-mo trial of Rx with tolbutamide on the growth and glucose homeostasis of children with CF to test the hypothesis that increased growth and LBM accretion could be enhanced as a result of increased insulin secretion or enhanced insulin action.

MATERIALS AND METHODS

Subjects were recruited from the CF patient population at Children's Hospital. None of the subjects were diabetic or had evidence of glucosuria. Procedures and risks were explained to the subjects and their parents. Before participation, written informed consent was obtained from the subjects and their parents, and verbal assent was obtained from the children. The study was approved by the institutional review board for use of human subjects in research.

Design and treatment. Subjects were studied for three consecutive, 4-mo periods. The first period was a Pre-Rx period (mo 0 to 4). The second period was the Rx period (mo 4 to 8) in which the subjects consumed 750 mg of tolbutamide (Orinase; Upjohn

Co, Kalamazoo, MI) per day in two doses (500 mg in the morning and 250 mg in the afternoon). The final 4-mo period served as a Post-Rx period (mo 8 to 12).

Rx with tolbutamide was commenced at various times of the year (number of subjects beginning Rx during that period): January to March (2); April to June (0); July to September (6); and October to December (4).

Stature and weight. Throughout the study, subjects reported to the CSC in the fasted state once a month for the measurement of height and weight. Height was measured three consecutive times by the same technician by using a standardized position of the patient and a stadiometer (Holtain Unlimited, Crymmych, Wales, United Kingdom). For this technician, the average (±SD) coefficient of variation for three consecutive height measurements on six subjects was 0.097 ± 0.074% (range, 0.00 to 0.20%). Weight was measured by using a calibrated balance scale.

Body composition. LBM was assessed in two ways. By using the equations of Slaughter *et al.* (19), which relate measured LBM to measured height, we estimated LBM by the height measurements at mo 0, 4, 8, and 12. Rates of LBM accretion per 4 mo were calculated from the differences between mo 0 and 4 (Pre-Rx), mo 4 and 8 (Rx), and mo 8 and 12 (Post-Rx). We directly assessed LBM by using TBW measurements on seven subjects just before beginning Rx (mo 4) and at the end of Rx (mo 8). Subjects reported to the CSC, voided, were weighed, and had baseline samples of saliva collected. After baseline sampling, subjects consumed a premeasured quantity of ^{18}O-labeled water (0.6 g/kg) of $H_2{}^{18}O$ (12.1 atom percent; Iso-Tech, Inc., Miamisburg, OH); the tracer was analyzed in our laboratory to verify enrichment. At 2, 3, and 4 h after the administration of the labeled water, saliva was collected and analyzed for ^{18}O content with a Finnigan MAT Delta E gas isotope ratio mass spectrometer (Finnigan MAT, San Jose, CA). The reproducibility (mean ± SD) of triplicate saliva measurements of the isotopic abundance of water ($\delta\ ^{18}O$) at natural abundance was 5.402 ± 0.187 atoms per million atoms (coefficient of variation, 3.5%). For enriched saliva samples ($n = 6$), the comparable values were 61.316 ± 0.581 atoms per million atoms (0.95%). The isotopic enrichment was used to calculate TBW by using the formula of Schoeller *et al.* (20):

$$TBW = \frac{d}{MW} \times \frac{APE}{100} \times 18.02 \times \frac{1.0407}{R_{pdb} \times \Delta\delta_{pdb}\ ^{18}O}$$

where d is the dose of $H_2{}^{18}O$ ingested in grams, MW is the molecular weight of the $H_2{}^{18}O$, APE is the atoms percent enrichment of ^{18}O, 18.02 is the atomic weight of ^{18}O, 1.0407 is the O_2 isotopic fractionation between CO_2 and H_2O at 25°C, R_{pdb} is the ratio of ^{18}O to ^{16}O in the Pee Dee Belemnite standard, and $\Delta\delta_{pdb}\ ^{18}O$, in units of atoms per million atoms, is the ^{18}O enrichment over baseline in the saliva 3 h after tracer ingestion. LBM was calculated assuming that 75.2% of the LBM of children is water (21).

Dose response. At mo 0 and on 3 separate days, subjects reported to the CSC after an overnight fast and received either 0, 250, or 500 mg of tolbutamide. Ninety minutes after the dose of tolbutamide, subjects consumed a standardized liquid meal (Meritene; Sandoz Nutrition, Minneapolis, MN) containing 400 kcal/m^2, of which 36.0, 30.6, and 33.4% of the total calories were supplied by protein, fat, and carbohydrate, respectively. Blood samples were drawn at min 0, 5, 15, 30, 45, 60, 90, 120, and 180 after the meal. For the 250- and 500-mg tests, blood samples were also drawn at 90 (−90) and 30 min (−30) before ingestion of the meal; these times corresponded to 0 and 60 min posttolbutamide ingestion, respectively. Blood samples were analyzed for glucose (coupled enzyme assay with hexokinase and glucose-6-phosphate dehydrogenase; Instrumentation Laboratory, Inc., Lexington, MA), insulin (RIA; Corning, Medfield, MA), and C-peptide (RIA; Immunex, Carson City, NV). Total AUC was calculated by triangulation.

Twenty-four h profile of insulin. Subjects were admitted to the General Clinical Research Center at mo 0, before the Pre-Rx period, and again at the end of mo 8 when still receiving the Rx. Comparisons of peak insulin values and the 24-h integrated insulin area were made between the two periods. After an overnight fast, an indwelling catheter was placed in a forearm vein of the subject. During the next 24 h, blood samples were drawn every 60 min between mealtimes during the day, every 30 min after a meal for 1 h, and every 2 h during the night. Serum blood samples (total of 27) were analyzed for insulin.

IGF. Identical-volume aliquots of serum from each sample collected during the 24-h period of insulin profiling were pooled to ensure adequate sample volume. The pooled sample was analyzed for concentrations of IGF-I and IGF-II (Endocrine Sciences, Van Nuys, CA). IGF-I and IGF-II values were transformed to z scores to account for normal age-related differences. Data provided by Endocrine Sciences were used to make the transformations.

Dietary intake. For 4 d (Thursday through Sunday) during each of the three periods of the study, subjects recorded daily food intake. Instructions were given to each patient on how to keep accurate records, and the parents assisted the patients in identifying, quantifying, and recording the foods consumed. Dietary records were analyzed by using data from Bowes and Church (22). For each 4-d period, the average values of the daily intake of energy, carbohydrate, protein, and fat were calculated.

Statistical analyses. One-way ANOVA was used to examine changes in the growth rate of height, weight, and predicted LBM. Also, one-way ANOVA was used to examine the total AUC of glucose, insulin, and C-peptide for the dose effect of tolbutamide during the meal stimulation tests and for the estimates of daily macronutrient intake. The Scheffe multiple comparison test was used to locate significant difference when an ANOVA was significant. Paired *t* tests were used to evaluate before and after Rx values for LBM measured with ^{18}O-water and for the serum concentrations of the 24-h insulin, IGF-I, and IGF-II. A probability level of 0.05 was selected for statistical significance.

RESULTS

The physical characteristics of the subjects at the initiation of the study (*i.e.* at the beginning of the Pre-Rx period) are presented in Table 1. On average, the subjects were at the 19th percentile for height, the 8th percentile for weight, and were 86% of the median body weight for height age.

During the course of the Rx period (*i.e.* mo 4 to 8), one female subject moved from Tanner stage II to Tanner stage III and two male subjects moved from Tanner stage I to Tanner stage II. None of the other nine subjects advanced in maturation level.

Statural growth and weight change. We observed a marked change in linear growth associated with tolbutamide Rx. The effect of Rx on the linear growth rate is shown in Figure 1 for all 12 subjects. During the Pre-Rx observation period, the rate of growth (mean ± SEM) was 0.88 ± 0.20 cm/4 mo. With treatment, linear growth almost tripled to 2.58 ± 0.31 cm/4 mo ($p < 0.05$). The Post-Rx rate of linear growth was 2.12 ± 0.16 ($p > 0.05$ *versus* Rx period and $p < 0.05$ *versus* Pre-Rx period).

The subgroup in whom TBW was measured ($n = 7$) showed similar changes in growth as compared with the whole group and had a significant increase in linear growth during the Rx. Within this subgroup, multiple comparisons showed that linear growth rate during Pre-Rx (0.83 ± 0.88 cm/4 mo) was significantly less than during Rx (2.70 ± 0.97 cm/4 mo) and Post-Rx (2.25 ± 0.57 cm/4 mo) ($p < 0.05$).

Upon excluding the three subjects who changed Tanner stage, we still observed an increase in linear growth during the drug Rx ($n = 9$; 2.38 ± 0.37 cm/4 mo) that was greater ($p < 0.05$) than the Pre-Rx (0.84 ± 0.24 cm/4 mo) but not different ($p > 0.05$) from the Post-Rx control period (1.94 ± 0.18 cm/4 mo).

Weight increased significantly during all periods of the study

Table 1. *Descriptive data on individual subjects at Pre-Rx period**

Subject no.	Sex	Age (y)	Height (cm)	Weight (kg)	TS	Pwt/ht	CF score
1†	M	13.0	148.2 (16)	35.1 (10)	I	88.9	98
2†	F	11.7	136.5 (3)	23.4 (5)	III	74.3	57
3	F	11.1	146.6 (57)	29.1 (10)	I	76.6	70
4†	F	12.0	130.8 (0)	25.0 (5)	I	90.9	61
5	M	11.1	130.1 (2)	21.0 (5)	I	77.8	63
6†	M	11.0	143.5 (51)	32.7 (25)	I	92.1	91
7	F	12.2	142.6 (7)	31.8 (10)	I	88.3	78
8†	F	13.4	143.9 (1)	31.5 (5)	II	86.3	93
9	F	6.1	112.8 (32)	18.1 (25)	I	96.5	86
10†	M	11.5	139.0 (14)	28.6 (10)	I	95.3	NA‡
11	M	9.1	125.2 (9)	21.6 (5)	I	88.2	42
12†	M	11.1	130.1 (2)	22.0 (5)	I	81.5	88
Mean ± SEM		11.0 ± 1.9	135.8 ± 3.0	26.7 ± 1.6		86.4 ± 2.1	

* Values in parentheses indicate percentile scores. TS, Tanner staging [breast development for females (F), pubic hair for males (M)]; the only subjects to change Tanner stage during the study were subjects 1 (I to II), 6 (I to II), and 8 (II to III). Pwt/ht, (actual weight/median weight for height age) × 100; CF score, NIH score for CF.

† Subjects receiving ^{18}O-water tests.

‡ NA, x-ray not available for scoring.

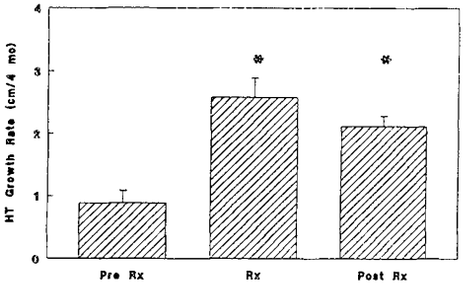

Fig. 1. Height (*HT*) growth rate (cm/4 mo) during Pre-Rx, Rx, and Post-Rx. Mean values are presented; *vertical lines* represent SEM. *, Rx and Post-Rx are greater than Pre-Rx ($p < 0.05$).

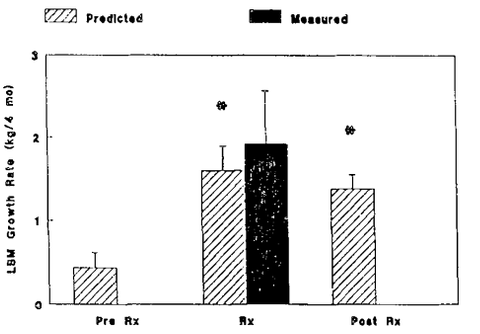

Fig. 2. LBM growth rate (kg/4 mo) predicted (*hatched bars*) during Pre-Rx, Rx, and Post-Rx. Measured rate during Rx (*solid bar*) is also presented. Mean values are presented; *vertical lines* represent SEM. *, Rx and Post-Rx are greater than Pre-Rx ($p < 0.05$).

Table 2. *Physical characteristics during study (mean ± SEM)**

	0 mo	4 mo	8 mo	12 mo
Height (cm)	135.8 ± 3.0[a]	136.6 ± 3.0[b]	139.2 ± 3.0[c]	141.3 ± 3.0[d]
Weight (kg)	26.7 ± 1.6[a]	27.7 ± 1.7[b]	29.2 ± 1.7[c]	29.9 ± 1.8[d]
FFW (kg)†		25.3 ± 1.9[a]	27.2 ± 1.9[b]	
Fat (kg)†		4.4 ± 0.6[a]	3.4 ± 0.5[a]	
% Fat†		14.7 ± 1.8[a]	10.9 ± 1.5[a]	

* 0 mo, measurement at beginning of Pre-Rx period; 4 mo, measurement at end of Pre-Rx period; 8 mo, measurement at end of Rx; 12 mo, measurement 4 mo after end of Rx; FFW, fat-free weight. Values with different superscripts are significantly different.

† Only seven subjects were tested for FFW, fat, and % fat.

($p < 0.05$). The rate of weight gain was not significantly different among the Pre-Rx (0.99 ± 0.26 kg/4 mo), Rx (1.50 ± 0.36 kg/4 mo), or the Post-Rx (0.69 ± 0.30 kg/4 mo) periods.

Similar to the group as a whole, the subgroup receiving the TBW test showed no difference in weight gain between Pre-Rx (1.16 ± 0.56 kg/4 mo), Rx (1.06 ± 0.38 kg/4 mo), and Post-Rx (0.59 ± 0.82 kg/4 mo) periods.

Removing the three maturing subjects did not affect the results for weight gain. For $n = 9$, values were: for Pre-Rx, 0.82 ± 0.33 kg/4 mo; for Rx, 1.56 ± 0.44 kg/4 mo; and for Post-Rx, 0.34 ± 0.30 kg/4 mo ($p > 0.05$).

Body composition. Absolute values for LBM and percent fat determined from TBW are presented in Table 2. The accretion of LBM (Fig. 2) as predicted from height was significantly greater during Rx and Post-Rx compared with the Pre-Rx observation period ($p < 0.05$). There was no difference in LBM accretion between the Rx and Post-Rx periods. The accretion of LBM as measured with ^{18}O-water confirms the predicted changes: there was no difference ($p > 0.05$) between the predicted rate (1.61 ± 0.29 kg/4 mo) and the measured rate (1.91 ± 0.65 kg/4 mo) ($r = 0.72$), and the mean value measured at month 8 at the end of Rx (27.2 ± 1.9 kg) was significantly higher than the Pre-Rx measurement (25.3 ± 1.9 kg).

Despite removing the three subjects who increased in Tanner staging, LBM measured with ^{18}O-water increased significantly ($p < 0.05$) during the Rx ($n = 4$; 21.6 ± 1.0 kg at month 4 *versus* 23.8 ± 1.5 kg at month 8; $p < 0.05$). No difference was observed between the measured increase in LBM and the increase in LBM predicted from height.

Fat mass determined from the TBW tests did not show a change (4.4 ± 0.6 kg before Rx *versus* 3.4 ± 0.5 kg after Rx; $p > 0.05$). The percent body fat determined by TBW was 14.7 ± 1.8% before Rx and 10.9 ± 1.5% after Rx ($p > 0.05$).

Acute glucose and hormonal response to tolbutamide. The glucose response to the meal stimulus was different under the conditions of no tolbutamide *versus* that seen after acute administration of tolbutamide (Fig. 3). The AUC (mean ± SEM) for glucose concentration was 902 ± 35, 767 ± 22, and 755 ± 33 mmol·min/L for 0, 250, and 500 mg of tolbutamide, respectively. The AUC for glucose during the 250- and 500-mg Rx were similar ($p > 0.05$); however, AUC for both Rx were significantly lower compared with the 0-mg treatment ($p < 0.05$).

The AUC (mean ± SEM) for the insulin response to the meal stimulus was 11 129 ± 1783, 12 199 ± 2254, and 16 675 ± 4997 pmol·min/L for 0, 250, and 500 mg of tolbutamide, respectively. There was no significant effect of tolbutamide Rx on AUC for insulin ($p > 0.05$).

No effect of tolbutamide was observed on C-peptide concentrations. The mean (±SE) areas were 68.8 ± 6.2, 69.0 ± 10.6,

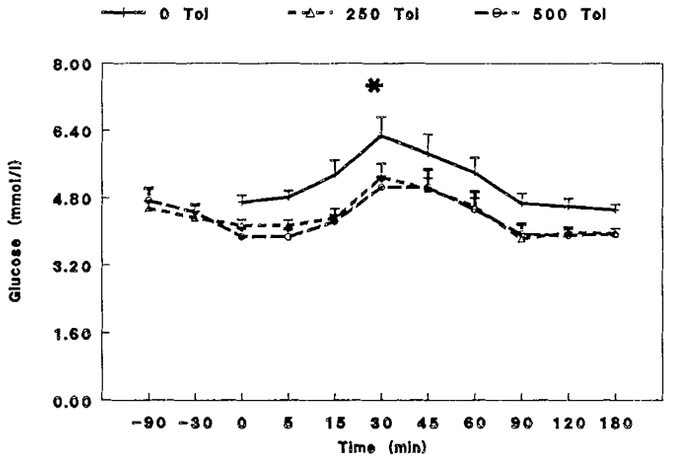

Fig. 3. Glucose response during meal stimulation test after administration of 0, 250, and 500 mg of tolbutamide (*Tol*). Mean values are presented; *bars* represent SEM. *, AUC for 250 and 500 mg are less than AUC for 0 mg of Tol ($p < 0.05$) ($n = 11$).

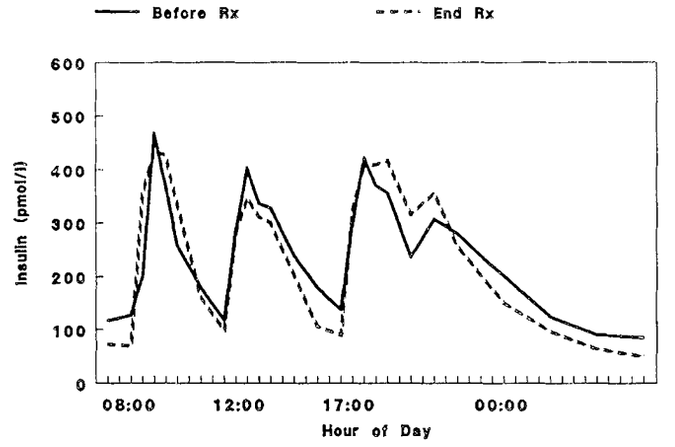

Fig. 4. Serum insulin levels during 24-h assessment before Rx (0 mg of tolbutamide/day) and after Rx (750 mg of tolbutamide/day). Mean values for 10 subjects are presented. No differences were found between AUC or peak levels at meal times ($p > 0.05$).

and 65.7 ± 13.0 nmol·min/L for tolbutamide doses of 0, 250, and 500 mg, respectively ($p > 0.05$).

Twenty-four h profile of insulin. All subjects had 24-h insulin profiles measured just before and again at the end of the 4-mo treatment period. There was no observed difference in insulin secretion patterns between the two tests (Fig. 4). The integrated insulin area before tolbutamide Rx was (mean ± SEM) 301 060 ± 26 483 pmol·min/L and after 4 mo of tolbutamide was 293 289 ± 37 126 pmol·min/L ($p > 0.05$). Similarly, there was no significant difference in the average postmeal, peak insulin response before therapy (487 ± 35 pmol/L) compared with the end of therapy (531 ± 69 pmol/L).

IGF. Absolute concentrations of IGF-I and IGF-II did not change significantly with the Rx, although the increase in IGF-I approached significance ($p = 0.10$). The concentrations (mean ± SEM; nmol/L) of IGF-I were 23.4 ± 3.1 and 28.5 ± 4.4 before and after Rx, respectively, and the concentrations (mean ± SEM; nmol/L) of IGF-II were 42.0 ± 2.8 and 41.2 ± 2.3 before and after treatment, respectively. Because IGF-I concentrations are dependent upon maturation level, we transformed IGF values to z scores based on the mean and SD score for each Tanner stage in normal children (Endocrine Sciences) (Fig. 5). The mean of the IGF-I z scores before Rx was significantly lower than 0 ($p < 0.05$); however, after Rx, the mean z score was not significantly different from 0, implying that the Rx may have normalized

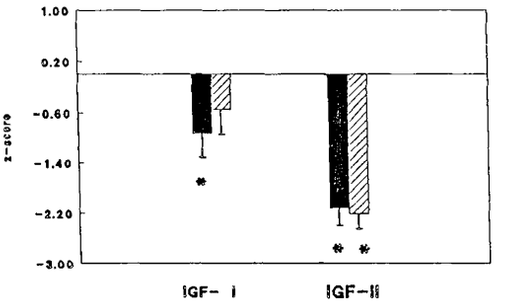

Fig. 5. IGF-I and IGF-II z score transformations before (*solid bars*) and at the end of (*hatched bars*) treatment. Means are presented; *vertical lines* represent SEM. *, Mean values are significantly less than 0 ($p < 0.05$).

Table 3. *Average (mean ± SEM) daily intake of energy and macronutrients per kg body weight for 4-d period during each period of study**

	Pre-Rx	Rx	Post-Rx
Energy (kcal/kg)	91.9 ± 21.2	84.7 ± 26.6	79.2 ± 26.8
Protein (g/kg)	3.17 ± 0.72	2.94 ± 0.92	2.80 ± 0.84
Carbohydrate (g/kg)	11.9 ± 2.6	10.6 ± 3.4	9.6 ± 4.1
Fat (g/kg)	3.57 ± 1.03	3.36 ± 1.19	3.25 ± 1.02

* No differences ($p > 0.05$) were found between periods for energy or any nutrient. $n = 9$.

IGF-I levels. IGF-II z scores were not different from each other, but both were significantly lower than 0 before and after Rx.

Dietary intake. Four-day prospective diet diaries were obtained 2 to 4 wk before starting tolbutamide, within the last 4 wk of therapy, and within 2 wk of completing the Post-Rx control period. Complete and accurate data were obtained on nine subjects. Analysis of the diary data in absolute units and per kilogram of body weight (Table 3) showed no difference between periods of the study for average daily intakes of energy, protein, carbohydrate, and fat ($p > 0.05$). On a per kilogram of weight basis, the CF patients consumed approximately 1.5 times the recommended dietary allowance of energy for children and 3 times the recommended dietary allowance of protein for children (23).

DISCUSSION

The purpose of this study was to determine if the linear growth rate and body composition of children with CF could be increased through stimulating an increase in insulin secretion and insulin action. The results suggest that the oral hypoglycemic agent tolbutamide increased linear growth and LBM accretion in excess of the control period. The linear growth rate during tolbutamide treatment was comparable to that seen during growth hormone Rx of growth hormone-deficient children (24). The finding of an increased accretion of LBM is particularly important because if most of the increase was in the skeletal muscle, increased skeletal muscle mass could effect a direct improvement in respiratory effort, cough, and pulmonary function as well as increased exercise capability with attendant improvement in the quality of life (3, 25–27).

The improved growth rate appears to be caused by the tolbutamide Rx. This is likely mediated by the anabolic effects of increased insulin action or possibly the direct effect of tolbutamide on IGF-I (28). During the study, no other events occurred nor were there other changes in therapy that would be expected to increase growth rate. Although it is possible that by lowering blood glucose, tolbutamide could stimulate appetite, the estimates of daily intake of energy and macronutrients were not different during the control periods or Rx period. Thus, within the limits of the study design, the improved growth would not

appear to be the consequence of increased caloric intake. Also, the increased growth rate does not seem to be explained by the change in maturation level. The change in two males from Tanner stage I to II would not distort to a great degree our results because most of the male growth spurt occurs between Tanner stages III and IV (29). Possibly, the data on the female subject who moved from Tanner stage II to III could bias the results because the growth spurt in females does occur between these two stages (29). Although this could be a significant confounding variable, analysis of weight gain, linear growth, and LBM without the data on the three subjects who showed some pubertal development did not change the statistical conclusions. During the Post-Rx period, the rate of weight gain was similar to that seen before therapy; however, the linear growth rate remained similar to that of the Rx period. This sustained improvement of linear growth after therapy concluded could indicate persistence of the drug effect. However, because the study design did not include a washout period after the treatment, further studies with alternative designs and controls will be required to answer this question.

Some children with CF have evidence of protein energy malnutrition and impaired growth (1, 3, 4, 30). In this regard, our subjects were not exceptional in that they showed low-percentile weights and heights (Table 1) and low percent body fat (initially 14.7%, Table 2) compared with values based on TBW measurements in normal children (≥19.5%) (21). However, our study design used convenience sampling as opposed to random sampling of the CF population. In addition, the families were allowed to know that the study was designed to evaluate the growth-promoting effects of tolbutamide. As a consequence, our sample is heavily biased towards those children who at the time of recruitment were short and who had shown a noticeable slowing of linear growth. These are serious threats to the external validity of the study if one would attempt to generalize these results to all children with CF. However, because this study was designed to evaluate the growth disorder of CF children, the results may be of particular importance and relevant only to the subgroup of CF children with the most severe growth problems.

For this group of CF children, we hypothesize that the poor growth is related to the general undernourished state among these subjects and that this is mediated by a relative insulin-deficient state. Insulin is a potent anabolic hormone, may be a direct stimulator of other growth factors necessary for normal growth (15–18), and appears to be necessary for normal expression of IGF-I mRNA (31, 32). Serum concentrations of insulin are below normal in many children with CF (10, 12, 13), and there is a high incidence of glucose intolerance in patients with CF (10, 11). Hypoinsulinemia in other conditions such as juvenile onset diabetes mellitus is associated with poor growth and short stature especially with inadequate insulin therapy (15, 33). A recent study suggests decreased survival in children with CF who develop glucose intolerance as opposed to those who do not show this complication (34).

Tolbutamide Rx in this study appeared to increase insulin action at peripheral sites, *i.e.* muscle and bone, but did not increase insulin secretion. We derive this conclusion from the dual observations that tolbutamide did effect lower blood glucose concentrations during the meal stimulation tests but had no effect on insulin concentration or C-peptide concentrations during the meal stimulation tests or the 24-h insulin secretion before and at the conclusion of tolbutamide therapy. The lack of an increase in insulin secretion after therapy was not totally unexpected as other researchers have reported that insulin secretion returns to Pre-Rx levels during chronic therapy with similar sulfonylurea agents (35, 36). Because insulin secretion did not appear to increase, we speculate that the increased growth and lean tissue deposition during tolbutamide therapy was caused by enhanced insulin action.

The mechanism of improvement in LBM during the tolbutamide therapy could relate to the indirect effects of insulin on carbohydrate utilization and production or to the direct effects of insulin on protein metabolism. Acute fasting tends to cause an increase in leucine oxidation, muscle protein degradation, and hepatic output of glucose; these effects may be related in part to decreased insulin secretion (37–39). Relative to fat as a source of energy, carbohydrate in the presence of insulin spares leucine during semistarvation (40) and glucose infusion in the postabsorptive state tends to suppress glucose production. Thus, if improved insulin action decreases endogenous glucose production, protein degradation might decrease. Insulin also had direct effects on protein turnover, both decreasing muscle protein degradation and increasing muscle protein synthesis (41–43).

Previous research supports our speculation that the observed growth-inducing effect of tolbutamide was related to enhanced insulin action. Craig *et al.* (44) found that insulin directly increased procollagen mRNA activity in cultured rat osteoblasts. Indirectly, insulin stimulates the production of receptors for IGF-I (31), which is known to stimulate growth at the epiphyseal plates (28, 45). Abnormally low IGF-I concentrations in malnourished children are associated with statural growth retardation (46, 47). Glibenclamide, another oral hypoglycemic agent, has been shown to normalize plasma IGF-I activity and increase skeletal growth in hypophysectomized rats (28). These investigators (28) reported that IGF-I activity was greater in those rats receiving the Rx *versus* hypophysectomized controls. In that study, the enhanced IGF-I levels may have been due to the glibenclamide's effect of increasing insulin, although the authors note that insulin levels did not return to normal with the treatment. This leaves open the possibility that the oral hypoglycemic agent directly affects IGF-I levels or bone growth.

In conclusion, nutritional status as indicated by stature and LBM was increased in slowly growing children with CF as a result of a tolbutamide administration. Insulin secretion was not increased, but the improved growth and physical stature of the subjects suggests that direct cellular effects of tolbutamide or indirect effects of tolbutamide on insulin responsiveness in the peripheral tissues improved. Although the findings are provocative, further studies are needed to determine if these effects are transient or are sustainable and to establish whether Rx with sulfonylurea drugs might constitute a valuable and safe adjunctive therapy for poorly growing children with CF.

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