Aspects of protein metabolism in children in acute and chronic illness

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Short-term protein intake and stimulation of protein synthesis in stunted children with cystic fibrosis

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ABSTRACT

Background: Stunted children with cystic fibrosis (CF) have less net protein anabolism than do children without CF, and the result is retarded growth in the CF patients. It is not known whether protein intake above that recommended by the Cystic Fibrosis Foundation would further stimulate whole-body protein synthesis.

Objective: We studied the effects of 3 amounts of protein intake on whole-body protein synthesis and breakdown by using isotopic infusion of [1-13C]valine and [15N2]urea in children with stable CF who required tube feeding.

Design: In 8 pediatric CF patients, we administered 3 randomly allocated isocaloric diets with normal (NP), intermediate (IP), and high (HP) amounts of protein (1.5, 3, and 5 g/kg/d, respectively) by continuous drip feeding during a 4-d period at 6-wk intervals. Each patient acted as his or her own control. On the fourth day of feeding, whole-body protein synthesis and breakdown were measured.

Results: Protein synthesis was significantly higher in the HP group (mean ± SEM: 1.78 ± 0.07 μmol/kg/min) than in the IP (1.57 ± 0.08 μmol/kg/min; p = 0.001) and NP (1.37 ± 0.07 μmol/kg/min; p < 0.001) groups. There were no significant differences in protein breakdown. Net retention of nitrogen was significantly higher in the HP group (12.93 ± 1.42 μmol/kg/min) than in the IP (7.61 ± 1.40 μmol/kg/min; p = 0.01) and HP (2.48 ± 0.20 μmol/kg/min; p < 0.001) groups.

Conclusion: In stunted children with CF requiring tube feeding, the highest stimulation of whole-body protein synthesis was achieved with a short-term dietary protein intake of 5 g/kg/d.
INTRODUCTION

Cystic fibrosis (CF) is the most common life-threatening recessive inherited disease in whites. The carrier frequency is 1 in 22–28 in whites, and the incidence is 1 in 2000–3000. Approximately 30,000 persons are affected in the United States (1). In the past 10–15 yrs, the mean survival of CF patients has increased significantly, from 20 to 34 yrs, as a result of improved medical care (2). One of the major factors contributing to this improvement is a greater emphasis on optimal nutrition. It has been shown that patients with better nutritional status have improved linear growth and maintain better pulmonary function and exercise tolerance (3–5). In addition, malnutrition has been shown to have adverse effects on respiratory muscle strength, ventilator drive, and immune defense mechanisms (6, 7).

Historically, the literature on nutritional strategies in CF has focused primarily on energy and fat intakes (8–12). In CF patients with a forced expiratory volume in 1 sec (FEV₁) > 85% predicted, the CF gene has no significant effect on resting energy expenditure, whereas, in those with FEV₁ < 85% predicted, there is a curvilinear increase in resting energy expenditure (13). For children with CF, an energy intake of 120–150% of the recommended dietary allowance (RDA) for healthy persons is currently recommended, including a high-normal fat intake of 35–40% of energy (14). It is well known that feeding enhances both whole-body and organ-specific protein synthesis in humans (15). However, there has been little study of the optimal amounts of dietary protein intake in pediatric CF patients.

At the time of a diagnosis of CF, severe protein deficits can already be present, and they result in lean body mass wasting or stunting of linear growth (16). The nutritional status is significantly worse in children diagnosed when symptomatic than in those diagnosed by neonatal screening (17). In general, protein deficits can result from increased protein breakdown, decreased protein synthesis, or both. Holt et al (18) found that children with CF with pulmonary infections have protein synthesis rates that are 50% lower than those in healthy controls and 43% lower than those in children with CF with chronic but stable pulmonary disease (p < 0.001). Conversely, in the same study, they found significantly greater whole-body protein breakdown (WBPB) in a group of stable CF patients than in an infected group of control subjects (=250% and 170%, respectively; p < 0.01) (18). During pulmonary infection, protein synthesis and net protein deposition can be enhanced with short-term nutritional supplementation (19, 20). Kien et al (21) studied the effects of enhanced feeding (protein intake: 4 g/kg/d) in stable pediatric CF patients and control subjects after a 12-hrs fast, and they found a 23% increase in protein synthesis in the fed state in the CF group (p = 0.058) and no change in WBPB. Under the same circumstances, WBPB increased 34% in the
healthy control group ($p = 0.001$), which indicated that feeding may affect protein turnover differently in children with stable CF and in healthy subjects.

We have been unable to identify any controlled studies that have investigated the effects of different dietary protein intakes on protein synthesis and breakdown in chronic and stable pediatric CF patients. The current recommended protein intake is 1.0–1.5 g/kg/d (22).

The aim of this study was, by using the isotopic infusion of $[1^{13}\text{C}]$valine and $[^{15}\text{N}_2]$urea, to ascertain the dietary protein intake that corresponded to a maximal rate of whole-body protein synthesis in pediatric CF patients with chronic but stable pulmonary disease.

**SUBJECTS AND METHODS**

**Subjects**

Children with a diagnosis of CF, confirmed with the use of a pilocarpine iontophoresis sweat chloride test, a CF gene mutation analysis, or both, were recruited from the pediatric outpatient clinics of 2 academic medical centers and one teaching hospital in the northwest part of the Netherlands. Eight patients were eligible, and all agreed to be enrolled. Inclusion criteria were age 7–12 yrs, prepubertal status, mild lung disease (FEV$_1$ > 75%), nasogastric or percutaneous endoscopic gastric feeding tube in situ, and no weight loss during the previous 30 days (23). Exclusion criteria were diabetes mellitus, clinical signs of intercurrent pulmonary infection (i.e., tachypnea > 30 breaths/min, increased coughing, or rectal temperature of $\geq 38.5$ °C), and the use of antibiotics other than chemoprophylaxis $\leq 14$ days before the beginning of the protocol. Home medications (i.e., bronchodilators, mucolytic agents, or pancreatic enzyme replacement therapy) were continued.

All procedures were explained to the subjects and their parents, and written informed consent was obtained from all subjects. The Medical Ethics Committee of the Academic Medical Center, Amsterdam, approved the study protocol.

**Study design and procedures**

We conducted a prospective, randomized, single-blinded, crossover trial of 3 isoenergetic diets containing 1.5 [normal-protein (NP)], 3 [intermediate-protein (IP)], and 5 [high-protein (HP)] g protein/kg/d in children with CF; each patient served as his or her own control subject. Endpoints were whole-body protein synthesis and WBPB and net nitrogen retention obtained by using a stable isotope technique.
At enrollment, a medical history was obtained and physical examination performed, including height, weight, temperature, and FEV₁, which was measured with a MasterScreen Paed (Viasys Healthcare, Bilthoven, the Netherlands). At the start of the study, oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were continuously measured for 30 min by indirect calorimetry and by using a ventilated hood system (Vmax model 2900; SensorMedics, Anaheim, CA). The energy intake of each diet was set at 200% of the patient’s individual baseline resting energy expenditure [i.e., ≈120% of the recommended dietary allowance for CF (24)]. During a 4-day period, the liquid study diet was administered via a gastric tube 24 hrs/day with the use of a feeding pump (Kangaroo 324; Sherwood Medical, St Louis). The diets consisted of a mixture of carbohydrate powder (Fantomalt), fat emulsion (Solagen), and protein powder (Protifar; all products of Nutricia, Zoetermeer, the Netherlands) and additional household salt dissolved in a body weight–related volume of water. Because of the continuous drip feeding, the carbohydrate intake was at a constant rate of 5 mg glucose/kg/min. The protein intake was varied per diet (1.5, 3, and 5 g protein/kg/d for the NP, IP, and HP diets, respectively), and the remaining nonprotein energy was supplied by fat. In addition, the children were allowed to drink nonenergetic beverages ad libitum. The sequence of the diets was randomly allocated, provided that within the study group all possible sequential combinations were assigned. The patients and their caretakers were blinded to the composition of the formula.

On the fourth day, a catheter was inserted antegradely in a deep antecubital vein of each arm. One catheter was used for sampling arterialized blood by using a heated (60 °C) hand box. At t = -5 min (0825), baseline blood and breath samples were taken for determination of background isotopic enrichments. At t = 0, via the second catheter in the opposite arm, primed continuous intravenous infusions of [1-13C]valine, at a rate of 0.153 μmol/kg/min (prime 9.1 μmol/kg), and [15N₂]urea, at a rate of 0.28 μmol/kg/min (prime 200 μmol/kg), were started, and a prime of NaH¹³CO₃ 4.2% was administered (2.1 μmol/kg) (all: Cambridge Isotopes, Cambridge, MA). The isotopes were dissolved in normal saline and infused by using a Perfusor Secura FT infusion device (B Braun Melsungen AG, Melsungen, Germany), sterilized by passage through an 0.20-μm millipore filter (Minisart; Sartorius AG, Göttingen, Germany). All infusions were tested for pyrogenicity by the hospital pharmacy.

After an equilibration period of 330 min of stable isotope infusion, 4 blood samples were collected at 10-min intervals (at 330, 340, 350, and 360 min), immediately stored on ice, and subsequently centrifuged at 1860 x g for 10 min at 4 °C. After separation, plasma was stored at -20 °C until analysis of isotopic enrichment of valine, α-ketoisovalerate (KIV), and urea (at t = 360 min). Plasma concentrations of insulin, glucagon, cortisol, catecholamines, and free fatty acids (FFAs) were measured.
During the experiment (8 hrs), tube feeding of an equivalent of one-third of the total daily dietary intake was continued. The entire protocol was repeated twice for each child, so that, at the end of all experiments, each child had been exposed to diets containing 1.5, 3, and 5 g protein/kg/d. Between these study diets, the children resumed their regular diet for a 6-week period.

**Assays**

Valine and urea were isolated on a cation exchange column (AG50W-X8; Bio-Rad Laboratories Inc, Hercules, CA) after precipitation of the plasma proteins with trichloroacetic acid. The proteins were eluted with ammonia and evaporated to dryness. Valine was derivatized to the N(O,S)-methoxycarbonylmethyl derivative as described by Hušek (25). After extraction with chloroform, an aliquot was injected into a gas chromatography-combustion-isotope ratio mass spectrometry system [(IRMS) HP 6890 series GC system; Hewlett-Packard, Palo Alto, CA; and Finnigan Deltaplus IRMS; Finnigan-MAT, Bremen, Germany]. Separation was achieved on a CP-SIL 19CB capillary column (25 m x 0.32 mm x 0.2 μm; Varian, Middelburg, the Netherlands). After combustion of the GC effluent to carbon dioxide, the $^{13}$C-enrichment of valine was measured by using IRMS. Data acquisition and delta calculations were performed by using THERMOFINNIGAN ISODAT NT software (version 0.144; Finnigan-MAT). Urea was measured as the bis-trimethylsilyl derivative described by Matthews et al (26). Isotopic enrichment was measured on a gas chromatography-mass spectrometry system (HP6890 series GC system and 5973 Mass Selective Detector; Hewlett-Packard) equipped with a J&W ScientificDB17 capillary column (30 m x 0.25 mm x 0.25 μm; Agilent Technologies, Palo Alto, CA). Selected ion monitoring (i.e., electron impact ionization), data acquisition, and quantitative calculations were performed by using CHEMSTATION software (version D.01.02.16; Agilent Technologies, Palo Alto, CA). The bistrimethylsilyl derivative was monitored at a mass-to-charge ratio (m/z) of 189 for urea and m/z of 191 for $[^{15}\text{N}]$urea. The ratio of urea tracer to tracee was calculated as described by Patterson et al (27). The $[^{1-13}\text{C}]\alpha$-KIV enrichment was determined in the O-tbutyldimethylsilyl-quinoxalinol derivative according to the method of Kulik et al (28). The same gas chromatography-mass spectrometry system was used as for the urea analysis. The KIV derivative was monitored at m/z 245 for KIV and m/z 246 for $[1-13\text{C}]$KIV. KIV tracer:tracee was calculated as described by Patterson et al (27).

Plasma insulin concentrations were ascertained by using an enzyme immunoassay on a Immulite analyzer (DPC, Los Angeles) with an intraassay CV of 3.5–6%, an interassay CV of 7.5–9%, and a detection limit of 15 pmol/L. Glucagon was measured by radioimmunoassay
High protein intake in chronic illness

(Linco Research, St Charles, MO) with an intraassay CV of 3–5%, an interassay CV of 9–13%, and a detection limit of 15 ng/L. Cortisol was measured by enzyme immunoassay on an Immulite analyzer with an intraassay CV of 2–4%, an interassay CV of 3–7%, and a detection limit of 50 nmol/L. Serum FFAs were measured by using an enzymatic method (NEFAC; Wako Chemicals GmbH, Neuss, Germany) with an intraassay CV of 2–4%, an interassay CV of 3–6%, and a detection limit of 0.02 mmol/L.

During the final 30 min (330–360 min) of isotope infusion, VO$_2$ and VCO$_2$ were measured by using indirect calorimetry. Concurrent breath samples were taken at 0, 60, 150, 240, 270, 300, 330, and 360 min for enrichment of 13CO$_2$ (BreathMATplus; Finnigan-MAT) to calculate total valine oxidation.

**Calculations and statistical analysis**

Dietary effects on whole-body protein synthesis ($S$) were studied by using 2 independent indicators: nonoxidative disposal of valine and net nitrogen retention in the body (29). The rate of appearance (Ra) of valine in plasma and valine oxidation ($\mu$mol/kg/min) were calculated according to standard equations (30–33).

To calculate the rate of $S$, which is equivalent to the rate of nonoxidative disposal of valine (NODVal; $\mu$mol/kg/min), valine oxidation was subtracted from the rate of appearance of valine (Ra$_{val}$):

$$ S = NODVal = Ra_{val} - \text{valine oxidation} \quad [1] $$

Endogenous valine breakdown ($B$) ($\mu$mol/kg/min) was calculated by subtracting exogenously administrated valine from the total RaVal:

$$ B = Ra_{val} - \text{Val(diet)} = \text{endogenous Ra}_{val} \quad [2] $$

Net valine deposition (anabolism) ($\mu$mol/kg/min) in the body was calculated by subtracting $B$ from $S$:

$$ \text{Net valine deposition} = S - B \quad [3] $$

From the urea data [Ra of urea (Ra$_{ur}$)], net nitrogen ($N$) retention ($S$) was calculated as

$$ S = \text{Net } N \text{ retention} $$

$$ = \text{dietary } N - (2 \times Ra_{ur}) \mu\text{mol/kg/min} \quad [4] $$
Repeated-measures analysis of variance was used to establish differences in these variables at normal, intermediate, and high dietary protein intakes. The analysis was performed by using the mixed procedure of SAS for WINDOWS statistical software (version 5.0.2195; SAS Institute Inc, Cary, NC) with compound symmetry covariance structure and the restricted maximum likelihood estimation method. \( P < 0.05 \) was considered significant. Data are presented as means ± SEMs.

RESULTS

Patients

Baseline characteristics of the patients are shown in Table 4.1. The patients were stunted mainly in linear growth (mean SD = -1.1), whereas their weight-for-height was normal (34).

Valine data

The individual data for the rate of appearance of valine and valine oxidation are shown in Table 4.2. Valine kinetics showed a significant, progressive increase of nonoxidative disposal with increasing dietary protein intake of 1.37 \( ± \) 0.07 \( \mu \text{mol/kg/min} \) in the NP, 1.57 \( ± \) 0.08 in the IP, and 1.78 \( ± \) 0.07 in the HP diets (Table 4.3). There was no significant change in the endogenous rate of appearance of valine between the 3 diets.

Table 4.1 Baseline characteristics of patients\(^a\)

<table>
<thead>
<tr>
<th>Patient and sex</th>
<th>Age yrs</th>
<th>Height(^b) cm</th>
<th>Weight(^b) kg</th>
<th>Best FEV(^c) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, M</td>
<td>12.0</td>
<td>149.5 (-1)</td>
<td>40.1 (0.5)</td>
<td>75</td>
</tr>
<tr>
<td>2, M</td>
<td>7.9</td>
<td>130.0 (-0.5)</td>
<td>29.5 (1)</td>
<td>106</td>
</tr>
<tr>
<td>3, M</td>
<td>11.5</td>
<td>138.0 (-2)</td>
<td>26.7 (-2)</td>
<td>90</td>
</tr>
<tr>
<td>4, M</td>
<td>9.2</td>
<td>131.0 (-1.5)</td>
<td>38.1 (2)</td>
<td>86</td>
</tr>
<tr>
<td>5, F</td>
<td>10.7</td>
<td>136.5 (-1.5)</td>
<td>26.7 (-1)</td>
<td>97</td>
</tr>
<tr>
<td>6, F</td>
<td>10.7</td>
<td>137.0 (-1.5)</td>
<td>27.7 (-1)</td>
<td>95</td>
</tr>
<tr>
<td>7, M</td>
<td>7.6</td>
<td>133.0 (0.5)</td>
<td>30.7 (1)</td>
<td>95</td>
</tr>
<tr>
<td>8, M</td>
<td>9.6</td>
<td>121.0 (-2.5)</td>
<td>25.1 (1)</td>
<td>88</td>
</tr>
<tr>
<td>Mean</td>
<td>9.5</td>
<td>134.5 (-1.1)</td>
<td>30.6 (0.2)</td>
<td>91.5</td>
</tr>
</tbody>
</table>

\(^a\)FEV\(_1\), forced expiratory volume in 1 second. \(^b\)Anthropometric data are shown as absolute numbers with SD scores in parentheses, according to Dutch growth charts generated by the 4th Nationwide Dutch Growth Study, 1997 (34). \(^c\)Percentage of predicted value.
**Urea data**

There was a significant increase in urea production (rate of appearance) with increasing dietary protein load: 4.43 ± 0.09, 7.59 ± 0.73, and 12.38 ± 0.65 μmol/kg/min in the NP, IP, and HP diets, respectively (Table 4.2). This increase was significantly less than the increase in protein intake, which resulted in net nitrogen retention with increased protein intakes: 2.48 ± 0.20, 7.61 ± 1.40, and 12.93 ± 1.42 μmol/kg/min in the NP, IP, and HP diets, respectively (Table 4.3).

**Hormone data**

There were no significant differences in the plasma concentrations of insulin, cortisol, and FFAs between the diets (Table 4.4). Only the plasma concentration of glucagon in the HP diet was significantly different from that in the NP and IP diets (82 ± 4, 65 ± 5, and 71 ± 4 ng/L, respectively).

**Table 4.2  Individual data for rate of appearance (Ra) of valine, valine oxidation, and Ra of urea**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ra val</th>
<th>Valine oxidation</th>
<th>Ra urea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NP</td>
<td>IP</td>
<td>HP</td>
</tr>
<tr>
<td>1</td>
<td>1.86</td>
<td>2.42</td>
<td>2.82</td>
</tr>
<tr>
<td>2</td>
<td>1.73</td>
<td>2.46</td>
<td>3.14</td>
</tr>
<tr>
<td>3</td>
<td>2.26</td>
<td>2.70</td>
<td>3.35</td>
</tr>
<tr>
<td>4</td>
<td>1.63</td>
<td>1.94</td>
<td>2.54</td>
</tr>
<tr>
<td>5</td>
<td>1.97</td>
<td>2.44</td>
<td>3.16</td>
</tr>
<tr>
<td>6</td>
<td>1.99</td>
<td>2.22</td>
<td>3.26</td>
</tr>
<tr>
<td>7</td>
<td>1.66</td>
<td>2.38</td>
<td>2.55</td>
</tr>
<tr>
<td>8</td>
<td>1.77</td>
<td>2.56</td>
<td>2.97</td>
</tr>
<tr>
<td>Mean</td>
<td>1.86</td>
<td>2.39</td>
<td>2.97</td>
</tr>
<tr>
<td>SEM</td>
<td>0.07</td>
<td>0.08</td>
<td>0.11</td>
</tr>
</tbody>
</table>

aN, normal-protein diet (1.5 g/kg/d); IP, intermediate-protein diet (3.0 g/kg/d); HP, high-protein diet (5.0 g/kg/d). Values in a row under each section heading with different superscript symbols are significantly different, p ≤ 0.01 (repeated-measures ANOVA). bPercentage of flux in parentheses.
The patients in our study were stunted predominantly in linear growth, whereas their weight-for-height was normal. This observation is in agreement with a large cross-sectional survey of > 3000 pediatric and adolescent CF patients in the United Kingdom, which showed a progressive, parallel, downward slope of SD curves for both length and weight as compared with the population without CF (35). In addition, during the past 5 yrs, the patient registry

### Table 4.3  Relative contribution of nonoxidative disposal of valine and endogenous rate of appearance (Ra) of valine to net valine fluxa

<table>
<thead>
<tr>
<th>Diet</th>
<th>NP</th>
<th>IP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/kg/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine flux</td>
<td>1.86 ± 0.07 (100)</td>
<td>2.39 ± 0.08 (100)</td>
<td>2.97 ± 0.11 (100)</td>
</tr>
<tr>
<td>Valine oxidation</td>
<td>0.49 ± 0.02 (26.3)</td>
<td>0.82 ± 0.03 (34.3)</td>
<td>1.19 ± 0.05 (40.1)</td>
</tr>
<tr>
<td>Nonoxidative disposal of valine</td>
<td>1.37 ± 0.07 (73.7)</td>
<td>1.57 ± 0.08 (65.7)</td>
<td>1.78 ± 0.07 (59.9)</td>
</tr>
<tr>
<td>Endogenous Ra of valine</td>
<td>1.26 ± 0.07 (67.7)</td>
<td>1.20 ± 0.11 (50.2)</td>
<td>1.05 ± 0.15 (35.4)</td>
</tr>
<tr>
<td>Valine balance</td>
<td>0.11 ± 0.03</td>
<td>0.37 ± 0.05</td>
<td>0.73 ± 0.12</td>
</tr>
<tr>
<td>Net nitrogen retention</td>
<td>2.48 ± 0.20</td>
<td>7.61 ± 1.40</td>
<td>12.93 ± 1.42</td>
</tr>
</tbody>
</table>

*aAll values are mean ± SEM; percentage of flux in parentheses. NP, normal-protein diet (1.5 g/kg/d); IP, intermediate-protein diet (3.0 g/kg/d); HP, high-protein diet (5.0 g/kg/d). Values in a row with different superscript symbols are significantly different, p ≤ 0.01 (repeated-measures ANOVA).

### Table 4.4  Plasma concentrations of hormones and free fatty acidsa

<table>
<thead>
<tr>
<th>Diet</th>
<th>NP</th>
<th>IP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>86 ± 7</td>
<td>93 ± 9</td>
<td>123 ± 26</td>
</tr>
<tr>
<td>Glucagon (ng/L)</td>
<td>65 ± 5*</td>
<td>71 ± 4*</td>
<td>82 ± 4*</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>173 ± 23</td>
<td>192 ± 42</td>
<td>187 ± 22</td>
</tr>
<tr>
<td>FFA (pg/mL)</td>
<td>0.11 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.09 ± 0.02</td>
</tr>
</tbody>
</table>

*aAll values are mean ± SEM. NP, normal-protein diet (1.5 g/kg/d); IP, intermediate-protein diet (3.0 g/kg/d); HP, high-protein diet (5.0 g/kg/d); FFA, free fatty acid. Values in a row with different superscript symbols are significantly different, p < 0.01 (repeated-measures ANOVA).

**DISCUSSION**

The patients in our study were stunted predominantly in linear growth, whereas their weight-for-height was normal. This observation is in agreement with a large cross-sectional survey of > 3000 pediatric and adolescent CF patients in the United Kingdom, which showed a progressive, parallel, downward slope of SD curves for both length and weight as compared with the population without CF (35). In addition, during the past 5 yrs, the patient registry
annual data reports (23,000 patients) of the American Cystic Fibrosis Foundation showed, for persons of both sexes aged 0–20 yrs, an average height at approximately the 30\(^{\text{th}}\)–35\(^{\text{th}}\) percentile and weight at the 40\(^{\text{th}}\) percentile until the age of 8 yrs, and this was followed by a gradual decline to the 25\(^{\text{th}}\)–30\(^{\text{th}}\) percentile; this indicates that most pediatric CF patients were stunted but had normal weight-for-height (36, 37).

In this study we showed that, in stunted pediatric CF patients with chronic but stable pulmonary disease, whole-body protein synthesis could be enhanced 30\% by increasing dietary protein intake to 5 g/kg/d. With the HP diet the rate of protein synthesis was significantly greater than that with the currently recommended NP diet. Protein breakdown, expressed as the endogenous rate of appearance of valine, did not change significantly. When reviewing relevant literature on the subject, we were unable to find a relation between either dietary protein or energy intake to WBPB (20, 38–40). We therefore concluded that the nonsignificant trend of a decrease of protein breakdown that we observed cannot be attributed to any dietary changes. The energy intake in the current study (72.6 ± 8.2 kcal/kg/d) is within the range of that cited in the aforementioned studies (20, 38–40).

So far, few studies have addressed the effect of different amounts of protein intake on rates of protein synthesis and breakdown in patients with CF. In all of those studies, protein intake was varied simultaneously with total energy intake, so that it remains unclear whether the results can be explained solely by different amounts of dietary protein. One study that compared the acute effects of 2 consecutive amounts of dietary protein intake (3.2 g protein/kg/d with 76 kcal/kg/d during 4 days, and 1.6 g protein/kg/d with 85 kcal/kg/d during 8 consecutive days) on whole-body protein metabolism in stunted adolescent and young adult CF patients did not find significant changes in protein synthesis or breakdown (20). However, in that study, 2 variables (both protein and energy intake) were altered at the same time, so that their specific relative contributions to the effect remained uncertain. A further confounder was the possibility of a carryover effect, because the study design did not correct for this possibility, and the result was a lack of difference in protein synthesis and breakdown rates.

In another study, nocturnal nutritional supplementation with a semielemental formula (1.14 g protein/kg/12 hrs, 32 kcal/kg/12 hrs, 70\% free amino acids (AAs), and 30\% small peptides), a nonelemental formula (2.49 g protein/kg/12 hrs, 45 kcal/kg/12 hrs, and 100\% casein), and a modified form of the nonelemental formula (1.14 g protein/kg/12 hrs, 32 kcal/kg/12 hrs, and 100\% casein) was compared (38). Although no significant changes in either protein synthesis or catabolism between the 3 nocturnal diets were observed in that study, net protein deposition was significantly (40\%, \(p < 0.05\)) greater with the nonelemental formula than with the modified nonelemental formula. However, the supplemental feeding with
the protein-rich formula was combined with a higher energy intake than was used with the lower-protein formula.

Several major long-term studies on the effects of 2 different amounts of protein intake in pediatric CF patients have been performed. In a cohort study over 2 consecutive yrs, Parsons et al (39) compared a nitrogen intake of 464 ± 16 mg/kg/d (which is equivalent to 2.9 g whole protein/kg/d) in the first y to a nitrogen intake of 608 ± 18 mg/kg/d (which is equivalent to 3.8 g whole protein/kg/d) in the second y. No changes were observed in either whole-body nitrogen flux or protein synthesis rates in the first y. The protein breakdown rates were significantly \((p < 0.05)\) lower during the second y, which resulted in greater net protein deposition and greater weight growth velocity \((p < 0.05)\). Height velocity was increased nonsignificantly \((p < 0.1)\), from a mean SD ± SEM of 1.03 ± 0.54 in the first y to a mean SD ± SEM of 0.26 ± 0.70 in the second y. Energy intake was significantly higher during the second y than during the first y \((82 ± 1\) and \(65 ± 2\ kcal/kg/d,\) respectively; \(p < 0.05)\). In an intervention study without control group, in which normal protein feeding via a gastrostomy tube was used to re-feed adolescent and adult CF patients, Vaisman et al found that body composition was restored with concomitant increase in energy expenditure after 1 y, without measurable changes in whole-body protein turnover (40). Finally, Shepherd et al (41) conducted a 2-y follow-up study that showed that both energy and protein supplementation \((≥120\%\) of RDA) in 10 stunted pediatric CF patients induced an initial (first mo) but nonsustained 50% stimulation of protein synthesis during nutritional supplementation. Nevertheless, this led to clinically significant catch-up growth in both weight and height within 6 mos, which continued for \(≥1\) y. In our short-term study, we found that protein synthesis and total-body protein content can be increased in pediatric CF patients by 4 days of isocaloric, high-protein intake. In long-term studies, the effect of extra protein will not show itself in protein kinetics measured with stable isotopes, because the changes in lean body mass develop only slowly under those circumstances, and stable isotope measurements in general are too insensitive to pick up the small difference between synthesis and breakdown. Long-term studies of the effects of extra protein should aim at effects on linear growth or body composition. Therefore, it would be very interesting to study long-term effects of isocaloric high-protein intakes on height and body composition in these children in a controlled, prospective study.

In the current study, there were no changes in the plasma concentrations of anabolic hormones between the diets. However, the nonsignificant amino AA-induced increase in plasma insulin concentration might have partly accounted for both the increase in protein synthesis and the decrease in protein breakdown (42). The only significant hormonal change we observed was the increased aminogenic glucagon plasma concentration with the HP diet. There is evidence
that glucagon has an attenuating effect on amino AA-induced increases in nonoxidative disposal of tracer isotopes and hence on the protein synthesis rate (43). Despite this effect, we observed significantly greater protein synthesis with the IP and HP diets. We concluded that the increased protein synthesis rates resulted from increased dietary protein intake.

In studies of valine kinetics, whole-body protein synthesis is calculated by subtracting valine oxidation from total flux. However, body retention of infused $^{13}$C can occur either in the form of nonexcreted $^{13}$CO$_2$ or as a result of fixation in metabolites other than carbon dioxide (44). This body retention of carbon dioxide will lead to an underestimation of valine oxidation and, inversely, to an overestimation of protein synthesis. $^{13}$CO$_2$ recovery factors obtained in postabsorptive states and euglycemic, hyperinsulinemic clamping studies vary from 0.67 to 0.82 (32, 45). Although there were no validated correction factors available for our study, we assumed the abovementioned effect to be equal for the 3 study diets. Moreover, we measured net nitrogen retention in the body as an independent variable of protein metabolism by studying urea kinetics.

The urea data supported the observations with the valine data. With the higher-protein diets, there was also a significant increase of $\approx$200% (a 3.1-fold rise) in net nitrogen retention in the body, despite the increase in urea production. Because dietary protein can be either oxidized only to urea and carbon dioxide or incorporated into the body, the increased positive nitrogen balance can be considered as an increase in protein synthesis in the body. Hence, these data show that both biochemical pathways (oxidation and incorporation of protein) were simultaneously yet unequally stimulated by increasing dietary protein intake.

In conclusion, our data support the hypothesis that, in stunted pediatric CF patients, short-term protein synthesis and thus net nitrogen retention in the body can be significantly increased by an HP (5 g protein/kg/d) diet. Whether these effects would be sustained with a prolonged diet, and thus lead to catch-up growth in height, requires further investigation.

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