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Original Article

Dietary essential amino acids are highly anabolic in pediatric patients with cystic fibrosis

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Abstract

Background: Current nutritional approaches have been partially successful in Cystic Fibrosis (CF). Essential amino acids mixtures with high Leucine levels (EAA) have anabolic properties in catabolic conditions, however data in CF are lacking.
Methods: On two days according a randomized crossover design, 15 pediatric CF patients ingested 6.7 g EAA versus mixture of total amino acids as present in whey. Whole body protein and Arginine metabolism (as EAA lack Arginine) were assessed by stable isotope methodology.
Results: Protein synthesis ($P<0.05$) but not protein breakdown was higher after EAA and 70% higher values for net anabolism ($P<0.001$)were found both in patients with and without nutritional failure. Arginine turnover was lower ($P<0.001$) and de novo Arginine synthesis tended lower ($P=0.09$) after EAA. Nitric oxide synthesis was not different.
Conclusions: CF patients are highly responsive to EAA intake independent of their nutritional status. Addition of Arginine to the EAA mixture may be warranted in CF.

Keywords: Cystic Fibrosis; Essential amino acids; Whole body protein anabolism; Arginine production; Nitric oxide synthesis; Nutritional failure

1. Introduction

Fat-free mass depletion, reflecting muscle mass loss, is present in 25–30% of children with Cystic Fibrosis [1,2], independent of their body mass index, and is associated with reduced lung function and bone mineral loss [1]. Previous studies in CF showed that oral supplementation of large amounts of calories to improve nutritional status is only partially successful and muscle gain is difficult to achieve in these patients [3,4]. Insight into the composition of dietary intake that is able to induce protein anabolism in children with CF is therefore of crucial importance to successfully counteract muscle wasting. Studies examining the effects of dietary proteins and amino acids in children with CF are limited, and their specific daily protein requirements are still unclear. A previous study showed that very high levels of dietary protein ($5\ g{\cdot}kg^{-1}{\cdot}d^{-1}$) are able to stimulate whole body protein synthesis in stable pediatric CF patients [5]. When taking into account the impaired protein digestion capacity of CF patients and the increased amino acid need for building muscle and acute phase proteins due to their inflammatory state [6], supplements with specific amino acids might be of clinical

Abbreviations: BMI, body mass index; CF, Cystic Fibrosis; cTTR, tracer–tracee ratios of amino acids corrected for their baseline value; DXA, dual-energy X-ray absorptiometry; EAA, essential amino acids; FEV_1, forced expiratory volume in 1 s; FFM, fat-free mass; FM, fat mass; FVC, forced vital capacity; NetPS, net protein synthesis; NO, nitric oxide; Q Arginine to Citrulline, nitric oxide production; Q Citrulline to Arginine, de novo Arginine production; Ra, rate of appearance; SE, standard error; TAA, balanced mixture of essential and non-essential amino acids; TTR, tracer tracee ratio; WbPB, whole body protein breakdown; WbPS, whole body protein synthesis.

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importance to increase the dietary nitrogen load in this patient population. In the past years, essential amino acids (EAA) have been shown to be solely responsible for the amino acid induced stimulation of muscle protein anabolism [7]. Furthermore, EAA are able to stimulate muscle protein synthesis to more than twice the extent as the same amount of a high quality (whey) protein [8]. When the proportion of leucine is increased to 40% in the EAA mixture, the rate of muscle protein synthesis is stimulated to an even greater extent [9]. Furthermore, leucine is known for its insulinotropic effect [10], which is of importance as a defect in the suppressive response of proteolysis to insulin is present in CF [11]. We therefore hypothesize that a leucine-enriched EAA mixture will stimulate protein anabolism in children with CF. Furthermore, CF is associated with reduced exhaled nitric oxide (NO) [12] despite the presence of chronic airway inflammation. Arginine is the sole precursor of nitric oxide (NO) and plays a key role in many other metabolic processes (i.e. protein synthesis, release of anabolic hormones, and modulation of immune function). It remains unclear to what extent dietary amino acid formulations are able to alter Arginine availability and NO production in CF and whether the EAA composition is optimal for CF patients.

In the present study, the acute anabolic effects of a leucine-enriched EAA mixture was examined in pediatric patients with CF and compared to that of a balanced mixture of essential and non-essential amino acids as present in whey protein. We also examined whether differences in the anabolic properties between the amino acid mixtures were associated with changes in arginine metabolism and NO synthesis rate. The data are required to support development of evidence based programs for nutritional support for pediatric CF patients using free amino acids to prevent or ameliorate muscle wasting and to reduce morbidity and mortality.

2. Materials and methods

2.1. Subjects

The study population consisted of 15 children with CF, age 10 to 21 years, and admitted to Arkansas Children's Hospital for antibiotic treatment of a pulmonary exacerbation. The subjects were clinically stable with no symptoms of pulmonary exacerbation anymore or other problems requiring use of drugs other than their usual medications. Furthermore, lung function of each subject at enrollment was back or was within 10% of baseline value (defined as the highest FEV_1 value obtained in the preceding year). All subjects were pancreatic insufficient based on 72 h fecal fat collection or fecal elastase level measurement. The patients were having one or more of the following pathogens: 8/15 (53%) Methicillin-resistant *Staphylococcus aureus* (MRSA), 6/15 (40%) Methicillin-sensitive *Staphylococcus aureus* (MSSA), 2/15 (13%) *Pseudomonas aeruginosa*, 1/15 (7%) *Mycobacterium avium*-intracellulare infection, 1/15 (7%) *Burkholderia cepacia*. Exclusion criteria included established diagnosis of diabetes mellitus and unstable metabolic diseases. Written informed consent/assent was obtained from all CF subjects and their parents in case the CF subject was <18 years, and the study was approved by the University of Arkansas for Medical Sciences Institutional Review Board (IRB#104738).

2.2. Composition of the nutritional supplements

In a double-blind and randomized crossover design, all CF subjects received on 2 subsequent study days one of the two nutritional supplements: 6.7 g high-leucine EAA mixture (containing 41% leucine)+15 g carbohydrates or 6.7 g balanced mixture of total (EAA and non-essential) amino acids+15 g carbohydrates. The subjects were given orally or enterally (when a feeding tube was present) one serving of each supplement. The content of the nutritional supplements is presented in Table 1. The amino acids and carbohydrate were provided by Ajinomoto Co. For an overview of the study design, see Fig. 1.

2.3. Anthropometric data and body composition

Body weight and height were measured by a digital beam scale and stadiometer, respectively. Height, weight and body mass index (BMI) percentiles of the CF subjects were calculated in accordance with the CF consensus report [13]. Whole body fat mass (FM) and fat-free mass (FFM) were obtained by dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500/Version 12.7.3.1 Bedford, MA), standardized for height [14] to obtain FFM-index and FM-index, and expressed as percentage of published reference data [15,16]. The DXA procedure was done once during hospital stay or the data were copied from subject's file when DXA was performed in the preceding month of the study as part of CF care.

2.4. Lung function

Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) was measured by spirometry (nSpire Health, Longmont, CO) and reference equations were used to calculate predicted values [17].

Table 1
Composition of the high-leucine essential amino acid (EAA) mixture and the isonitrogenous balanced mixture of total (essential and non-essential) amino acids.

Amino Acid	EAA (g)	Total AA (g)
Histidine	0.239	0.33
Isoleucine	0.614	0.31
Leucine	2.790	0.53
Lysine	1.069	0.65
Methionine	0.284	0.17
Phenylalanine	0.398	0.27
Threonine	0.751	0.33
Valine	0.580	0.36
Other amino acids	0	3.67 [a]
Total	6.726	6.726
Maltodextrin	15 g	15 g

[a] Other amino acids: alanine (0.46 g), arginine (0.41 g), aspartic acid (0.61 g), cystine (0.08 g), glutamic acid (0.96 g), glycine (0.31 g), proline (0.31 g), serine (0.28 g), and tyrosine (0.25 g).