Stimulated Nitric Oxide Production and Arginine Deficiency in Children with Cystic Fibrosis with Nutritional Failure

Mariëlle P. K. J. Engelen, PhD[1,3], Gulnur Com, MD[2], Yvette C. Luiking, PhD[1], and Nicolaas E. P. Deutz, MD, PhD[1,3]

Objective To determine whether upregulated whole body de novo arginine synthesis and protein breakdown are present as a compensatory mechanism to meet the increased demand for arginine and nitric oxide (NO) production in pediatric patients with cystic fibrosis (CF) and nutritional failure.

Study design In 16 children with CF, studied at the end of antibiotic treatment for a pulmonary exacerbation, and 17 healthy controls, whole body arginine, citrulline (Cit), and protein turnover were assessed by stable isotope methodology and de novo arginine synthesis, arginine clearance, NO synthesis, protein synthesis and breakdown, and net protein balance were calculated. The plasma isotopic enrichments and amino acid concentrations were measured by liquid chromatography–tandem mass spectrometry.

Results Increased arginine clearance was found in patients with CF ($P < .001$), whereas whole body NO production rate and plasma arginine levels were not different. Whole body arginine production ($P < .001$), de novo arginine synthesis, and protein breakdown and synthesis ($P < .05$) were increased in patients with CF, but net protein balance was comparable. Patients with CF with nutritional failure (n = 7) had significantly higher NO production ($P < .05$), de novo arginine synthesis, Cit production ($P < .001$), and plasma Cit concentration ($P < .05$) and lower plasma arginine concentration ($P < .05$) than those without nutritional failure (n = 9).

Conclusions Nutritional failure in CF is associated with increased NO production. However, up-regulation of de novo arginine synthesis and Cit production was not sufficient to meet the increased arginine needs leading to arginine deficiency. *(J Pediatr 2013;163:369-75)*.

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Low exhaled nitric oxide (NO) concentrations (fractional exhaled nitric oxide [FeNO]) are often found in patients with cystic fibrosis (CF)[1] despite the presence of airway inflammation. Several studies found positive correlations between pulmonary function and FeNO in CF,[1,2] suggesting that the reduced NO levels in CF airways contribute to functional changes of the airways. It is possible that diminished local or systemic arginine (Arg) availability is an important factor negatively affecting pulmonary NO production in CF. Increased conversion of Arg by the enzyme arginase in sputum[3] may account for substrate limitation for NO production in stable CF. If an increased need for Arg exists in stable patients with CF, Arg production should be upregulated to meet its needs. Plasma Arg levels are preserved in stable CF but reduced during an acute inflammatory exacerbation.[1,4] Arg is considered a conditionally essential amino acid, especially during growth and stressful metabolic conditions (ie, sepsis),[5] when Arg production from endogenous (de novo) Arg production (from citrulline [Cit]) and protein breakdown may not be sufficient and will reduce NO synthesis and muscle protein synthesis.[6] We have found that in several inflammatory states, alterations in Arg metabolism contribute to muscle wasting,[6] and that the presence of malnutrition reduces the response of Arg-NO metabolism to inflammation.[7] We hypothesize that an upregulated de novo Arg synthesis and a stimulated protein breakdown are present in stable patients with CF to meet the increased demand for arginase and NO production and that this is related to enhanced muscle protein wasting, particularly in those patients with nutritional failure. In the present study, we investigated whether whole body NO synthesis and (de novo) Arg production is increased in patients with CF.

ADMA	Asymmetric dimethyl arginine
Arg	Arginine
CF	Cystic fibrosis
Cit	Citrulline
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second
FFM	Fat-free mass
FM	Fat mass
NO	Nitric oxide
Orn	Ornithine
WbRa	Whole body rate of appearance

From the [1]Department of Geriatrics, Center for Translational Research in Aging and Longevity, University of Arkansas for Medical Sciences; [2]Department Pediatric Pulmonology, Arkansas Children's Hospital, Little Rock, AR; and [3]Department of Health and Kinesiology, Translational Research in Aging and Longevity, Texas A&M University, College Station, TX

Supported by the Arkansas Children's Hospital Research Institute and the Arkansas Biosciences Institute (major research component of the Tobacco Settlement Proceeds Act of 2000), a partnership of scientists from Arkansas Children's Hospital, Arkansas State University, the University of Arkansas for Medical Sciences, the University of Arkansas Division of Agriculture, the University of Arkansas, Fayetteville, and the National Institutes of Health (S10RR027047). Y. L. is an employee of Nutricia Advanced Medical Nutrition, Danone Research–Centre for Specialized Nutrition, Wageningen, The Netherlands. The authors declare no conflicts of interest.

 http://dx.doi.org/10.1016/j.jpeds.2013.01.005

Furthermore, we studied whether changes in whole body Arg and Cit metabolism in patients with CF with and without nutritional failure were associated with alterations in protein metabolism.

Methods

Sixteen pediatric subjects with CF admitted to Arkansas Children's Hospital for a pulmonary exacerbation and 17 healthy controls were studied. The patients with CF were studied in clinically stable condition during the last days of a 14-day intravenous antibiotic treatment course. Lung function (forced expiratory volume in 1 second [FEV_1]) was back or close to baseline values (highest FEV_1 in past year). Exclusion criteria included diabetes mellitus and unstable metabolic (ie, liver and renal) diseases. The healthy subjects were recruited from the local community. Written informed consent was obtained and the study was approved by the institutional review board.

Body weight was measured by using a digital beam scale, and height was measured by using a stadiometer; both were expressed as percentiles. Whole body fat mass (FM) and fat-free mass (FFM) of the patients with CF were obtained with dual-energy x-ray absorptiometry (Hologic QDR 4500/Version 12.7.3.1; Bedford, Massachusetts) and in healthy subjects with bioelectrical impedance spectroscopy (Xitron 4000B; Xitron Technologies, San Diego, California) and standardized for height to obtain FFM and FM index (percent). The anthropometric and body composition data were compared with published reference data.[8] Dietary intake was estimated by food frequency questionnaires. In the CF group, nutritional failure was defined as FFM index <5th percentile and/or body mass index <10th percentile (age ≤20 years).[8] FEV_1 and forced vital capacity were measured by spirometry (nSpire Health, Longmont, Colorado).[9]

Patients with CF were studied in their hospital room, and the healthy controls were examined on the metabolic ward after overnight fasting. For infusion of the stable isotopes (Table I; available at www.jpeds.com), an indwelling venous access port or antecubital vein was used. A blood sample was taken and the primed constant continuous tracer infusion was started with a calibrated pump. A second catheter for blood sampling was placed in a superficial hand vein and a hotbox was used to obtain arterialized-venous blood.[10] Infusion lasted for 2-3 hours with 3 blood samples in the last 30 minutes. Blood was treated as reported previously.[5] Analysis for enrichment and concentrations was done with liquid chromatography–electrospray ionization–mass spectrometry (QTrap 5500MS; AB Sciex, Foster City, California) with ExpressHT Ultra LC (Eksigent; AB Sciex) after derivatization with 9-fluorenylmethoxycarbonyl.[11] 9-Fluorenylmethoxycarbonyl was fragmented to obtain specific and high-sensitivity fragments. Whole body Arg, Cit, and protein turnover were assessed by stable isotope methodology, and de novo Arg synthesis, Arg clearance, protein synthesis and breakdown, and net protein balance were calculated. Plasma Arg and Cit fluxes were calculated from the isotope enrichment values of L-[guanidine-$^{15}N_2$]Arg and L-[ureido-^{13}C-$^{2}H_2$]Cit. NO synthesis was calculated by calculating the Arg-to-Cit flux: QCit × TTRCit (M + 1)/TTRArg (M + 2), where QCit is the plasma Cit flux, estimated from the infusions of the L-[ureido-^{13}C-$^{2}H_2$]Cit tracer; and TTRCit and TTRArg are the respective tracer:tracee ratios of L-[guanidino-^{15}N]Cit and L-[guanidino-^{15}N]Arg. All metabolic data were determined under steady-state conditions and subsequently calculated.[5]

Statistical Analyses

Results are expressed as mean ± SEM. The mean value of the measures of Arg and protein kinetics at the triple sample points was used. Data failing the normality or equal variance test were log-transformed where appropriate. One-way ANOVA was used to determine differences between the CF groups with and without nutritional failure and the control group, and Newman-Keuls was used for posthoc analysis. Unpaired Student *t* test was used to determine differences in clinical changes between the CF group with and without nutritional failure. The level of significance was set at $P < .05$. The statistical package within GraphPad Prism (version 5.04) and SPSS (version 20) (SPSS Inc, Chicago, Illinois) was used for data analysis.

Results

Age and height did not differ significantly between the CF groups with and without nutritional failure (Table II) but were lower in both groups compared with the control group ($P < .01$). Body mass index percentile was significantly lower in the CF group with nutritional failure compared with those without nutritional failure ($P < .01$). FFM index and FM index (as percent of normal value) were not significantly different between the CF without nutritional failure and the healthy control groups but were lowest in the CF group with nutritional failure ($P < .01$). FEV_1 was not different between the CF groups with and without nutritional failure, although forced vital capacity tended to be lower in the group with nutritional failure ($P = .06$).

Clinical Status and Dietary Intake in CF Subgroups

Absolute change of body weight (Table II) in the preceding 3 months before hospital admission was higher in the CF group with nutritional failure ($P < .05$) than in those with a normal nutritional status. Six patients with CF with nutritional failure (86%) and 2 (25%) with a normal nutritional status were characterized by weight loss before hospital admission. Weight change during hospital stay was not different between the groups. The numbers of exacerbations and hospital admissions in the preceding year before hospital admission were both significantly higher in the CF group with nutritional failure than in those with a normal nutritional status ($P < .05$). Dietary intake (energy measured in kcal, protein in g and percent, and carbohydrates and fat in percent) between enrollment and study days and before hospital admission was not