# Evolution of Pancreatic Function during the First Year in Infants with Cystic Fibrosis

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**Objective** To describe pancreatic function during the first year of life in infants diagnosed with cystic fibrosis (CF) using serial fecal elastase measurements.

**Study design** This was a longitudinal study of 82 infants diagnosed with CF through newborn screening. Monthly stool samples were sent to a central laboratory for fecal elastase measurements.

**Results** A total of 61 infants had an initial stool sample obtained at age <3.5 months and a final stool sample obtained at age >9 months. Twenty-six of 29 infants with a fecal elastase value <50 μg/g at study entry had a fecal elastase value <200 μg/g (the accepted cutoff value for pancreatic insufficiency) on all measurements during the year; all 29 had a value <200 μg/g at the end of the study. Of the 48 infants with initial fecal elastase value <200 μg/g, 13 had at least 1 fecal elastase value >200 μg/g but had a final stool fecal elastase value <200 μg/g; however, 4 infants with an initial fecal elastase value <200 μg/g ended the year with a value >200 μg/g. Eleven of 13 infants with an initial fecal elastase value of >200 μg/g still had a value >200 μg/g at the end of the first year.

**Conclusion** Infants with CF exhibit variability in fecal elastase values during the first year. Infants with a fecal elastase level of 50-200 μg/g at diagnosis should be treated with pancreatic enzyme replacement therapy, but fecal elastase should be remeasured at age 1 year to ensure that those with a falsely low value do not continue to receive pancreatic enzyme replacement therapy unnecessarily. Those with a fecal elastase value >200 μg/g initially can become pancreatic insufficient with time. *(J Pediatr 2013;162:808-12)*.

Over the past decade, newborn screening for cystic fibrosis (CF) has become universal in the US and is performed routinely in numerous countries worldwide. Newborn screening detects patients with CF who are both pancreatic insufficient (PI) and pancreatic sufficient (PS).[1] Although most infants with CF are PI and require exogenous pancreatic enzyme replacement therapy (PERT), a substantial number may be PS at birth only to become PI over time.[2] In the past, physicians diagnosed pancreatic insufficiency either based on clinical signs and symptoms, such as steatorrhea or poor growth, or with a 72-hour fecal fat test, measuring the coefficient of fat absorption. When diagnosed clinically, pancreatic insufficiency cannot be detected until the infant is in danger of nutritional compromise, and, without a proper test of pancreatic function, some infants with poor growth or loose stools may be misdiagnosed as being PI when in fact they are not. Measuring fat absorption is problematic, because the parents must accurately monitor fat intake and collect stool for 3 days while the child is not receiving PERT, making the test unpopular and potentially unreliable. Furthermore, there are little published normative data on fat absorption in the first months of life.

A simple, noninvasive means of testing for pancreatic function in CF is the fecal elastase test.[3-5] Pancreatic elastase is produced by acinar cells of the pancreas and is not degraded during transit through the bowel. The test is specific for human fecal elastase, meaning that the patient can continue receiving porcine-derived PERT when undergoing testing. The sample does not require refrigeration, and needs only a very small amount of stool collected from a single bowel movement. Fecal elastase is stable for weeks at room temperature. Monoclonal and polyclonal fecal elastase tests have demonstrated excellent positive predictive values for identifying PI patients, although the absolute fecal elastase value determined by the polyclonal method is higher than that determined with the monoclonal test.[6,7] The manufacturer of the commonly used polyclonal test suggests that a fecal elastase value >200 μg/g is consistent with pancreatic sufficiency,[8] whereas others have suggested that 180 μg/g is a more accurate cutpoint in patients with CF.[9]

The majority of previous studies of fecal elastase in patients with CF have been cross-sectional, with single samples obtained from patients of varying ages. A few studies have examined multiple samples from individual patients, but these

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| CF | Cystic fibrosis |
| DHA | Docosahexaenoic acid |
| PERT | Pancreatic enzyme replacement therapy |
| PI | Pancreatic insufficient |
| PS | Pancreatic sufficient |

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studies were small and did not focus on the first year of life,[10,11] or were retrospective.[12] We report the longitudinal evaluation of pancreatic function in patients with CF based on fecal elastase values measured during the first year of life, and provide information on the natural history of pancreatic function in these patients.

## Methods

The subjects of the present study were enrolled in a larger parent study designed to examine the effect of supplementing infant formula with docosahexaenoic acid (DHA) during the first year of life (ClinicalTrials.gov: NCT00530244). Parents of infants with confirmed CF as defined by a positive sweat test by pilocarpine iontophoresis or the presence of 2 known CF-causing mutations, were approached to provide informed consent. Infants who were exclusively formula-fed and were entered into this study by 54 days of age were eligible. The eligible infants were randomized to receive either standard infant formula without long-chain polyunsaturated fatty acids (Enfamil) or standard formula supplemented with arachidonic acid (0.31% of total fatty acids) and DHA (0.93% of total fatty acids). The formulas were isocaloric and contained the same total amount of fatty acids (Mead Johnson Nutrition, Evansville, Indiana). Parents were given containers and prepaid packaging and were asked to send a stool sample each month to the central laboratory at the Women and Children's Hospital of Buffalo, New York. The study was approved by the Institutional Review Boards at the participating sites.

Stool elastase testing was done by pancreatic elastase solid-phase enzyme linked immunosorbent assay (BIOSERVE Diagnostics, Rostock, Germany). This assay is based on a double-sandwich technique, applying 2 polyclonal antibodies recognizing several different epitopes on defined species and organ-specific human pancreatic elastase peptide sequences. The microplate is coated with antibodies directed against human pancreatic elastase that binds the pancreatic elastase contained in the patient samples or standards. The second antibody, labeled with biotin, then binds to the immobilized pancreatic elastase. Next, streptavidin-labeled horseradish peroxidase is added and binds to the biotin. The peroxidase oxidizes the substrate 3,3′,5,5′-tetramethylbenzidine. The reaction is stopped by the addition of $H_2SO_4$, and the oxidized 3,3′,5,5′-tetramethylbenzidine is measured photometrically at 450 nm.[8]

Baseline characteristics of the included and excluded infants were compared using the Fisher exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables. Linear mixed modeling was used to assess the effect of the intervention on fecal elastase levels. Fecal elastase values were modeled as a function of infant's age at laboratory testing, the treatment arm, and their interaction. No significant differences between the randomization groups were evident. To illustrate the changes in fecal elastase values over time, measurements were grouped into time windows (0.8-3.5, 3.6-5.6, 5.7-7.9, 8.0-10.0, and 10.1-13.6 months), and geometric means with SE bars were graphed over infant age for the 2 randomization groups. We examined groups depending on final fecal elastase values above or below Bioserv's recommended cutpoint between PI and PS of 200 μg/g. We also evaluated whether using a level 10% above or below this value affected characterization of the subject's pancreatic status, based on previous work by Walkowiak et al.[9]

## Results

Eighty-two infants were enrolled in the study; parents of 1 infant withdrew consent. Two or more stool samples were received from 77 infants, 1 of whom was excluded for not meeting the protocol-defined definition of CF. We limited the evaluable cohort to infants who provided their first sample before age 3.5 months and their final sample at age ≥9 months, to ensure that we could assess changes in fecal elastase occurring over the entire first year of life. This left a cohort of 61 infants, 53 of whom provided at least 8 stool samples (**Table**).

Twenty-eight infants (46%) were homozygous for the F508del mutation. Ten of the compound heterozygotes had genotypes frequently associated with pancreatic sufficiency, and 5 others had the F508del mutation identified on one allele along with an unidentified second mutation (but with an elevated sweat chloride level diagnostic of CF). It should be noted that there is no universal agreement on what constitutes a PS genotype; for example, 3 subjects were heterozygous for a severe mutation and G85E, a genotype associated with PI in 70-80% of the cases in one survey[13] but which has been described as a mild PS mutation by others.[14]

Pancreatic function, as measured by mean fecal elastase level over the first year of life, did not differ between the standard formula and study formula groups (**Figure 1**; available at www.jpeds.com). Thus, data from all infants regardless of treatment assignment were pooled for further analysis.

Of the 29 infants with an initial fecal elastase value <50 μg/g, 3 had a value >200 μg/g at some time during the first year of life, but all had a value <200 μg/g at age 1 year. Another 7 infants had an initial fecal elastase value of 50-100 μg/g. Thirty-two of the 36 infants with an initial fecal elastase value <100 μg/g also had a final fecal elastase value <100 μg/g, and only 1 infant had fecal elastase value consistent with PS (>200 μg/g) at the end of the study. This infant was homozygous for the 2789+5 G>A mutation, a mutation associated with PS approximately 40% of the time in infants heterozygous for this mutation.[15]

Greater variability was seen when assessing the 12 infants with an initial fecal elastase value of 100-200 μg/g. **Figure 2** provides a schematic representation of the variable fecal elastase results detected during the first year of life in the 48 infants with an initial fecal elastase value <200 μg/g. Although only 4 of these 48 infants with an initial fecal elastase value consistent with PI ended up with a value in the PS range, 13 others had at least 1 value >200 μg/g during the year. **Figure 3** shows the variability over the first year of life in a cohort of 8 infants who stood out for having an initial value <200 μg/g but fluctuating values