Published in final edited form as:

J Pediatr Gastroenterol Nutr. 2010 April ; 50(4): 441–446. doi:10.1097/MPG.0b013e3181b18308.

Fat Malabsorption in Cystic Fibrosis: Comparison of Quantitative Fat Assay and a Novel Assay Using Fecal Lauric/Behenic Acid

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Abstract

Objectives—The “gold standard” for the diagnosis of fat malabsorption, the 72 hour fat balance study, requires a three day collection to generate a coefficient of fat absorption (CFA). We hypothesized that, a new test using behenic acid (behenate test) as a nonabsorbable lipid marker may provide a facile means to assess fat absorption. The study proposed to answer two questions: 1) whether the “behenate test” correlated with the “gold standard” and 2) whether the CFA improved when taking pancreatic enzymes during meals instead of prior to them.

Methods—The study compared the “behenate test” with the gold standard in 15 cystic fibrosis patients during three arms which require 3–4 day hospitalizations: one taking pancreatic enzymes prior to meals, one taking pancreatic enzymes during meals, and one off of pancreatic enzymes.

Results—The mean CFA was 78.3% when pancreatic enzymes were taken during meals and 80.4% when pancreatic enzymes were taken prior to meals. Correlation between the CFA and the “behenate test” for collections during all 3 arms was $r^2 = 0.219$ ($p = 0.001$).

Conclusion—1) Timing of ingestion of pancreatic enzymes does not significantly alter the CFA. 2) Although the CFA correlates with the “behenate test”, the correlation is not robust enough to justify its replacement of the “gold standard.” It is unclear whether the poor correlation between tests relates to intermeal variability in fat excretion, or other factors; however the “behenate test” may be suitable as a screening test for detection of fat malabsorption.

Keywords

malabsorption; stool; cystic fibrosis; CFA

INTRODUCTION

Steatorrhea results from impaired digestion or absorption of dietary fats and it can be caused by multiple diseases including cystic fibrosis, chronic pancreatitis, cholestatic liver disease,

celiac disease, and inflammatory bowel disease(1). If untreated, fat malabsorption may result in malnutrition, growth failure, and deficiencies of fat-soluble vitamins A, E, D, and K with resultant skin and visual changes, neurologic deficits, osteoporosis/rickets and coagulopathy.

Currently the gold standard test to diagnose steatorrhea remains the 72 hour fat balance method, which is based on the premise that fat intake minus fat output equals fat absorbed (2). A coefficient of fat absorption, calculated from the intake/output data, is the standard value used to indicate malabsorption. This test is very time consuming and logistically difficult as it requires a three to five day stool collection and a complete dietary intake record. Additionally, most patients with fat malabsorption have diarrhea and therefore accurate and complete collection is difficult, particularly in infants and children. These limitations make the 72 hour fat balance method impractical in the clinical setting and stress the need for a facile, accurate test of fat malabsorption. Despite previous attempts to develop simpler methods than the 72 hour fat balance method, none have proven to be easier than and as reliable as the gold standard.

Preliminary investigations in mice and rats have provided evidence that the technique of administering a small amount of sucrose polybehenate is a potentially viable method for assessing stool fat without collecting 72 hours of stool (3). Early work with humans suggested that either linoleic or linolenic acid would be excellent fatty acids for comparison; however, it was found that dietary contamination and bacterial degradation made them unsatisfactory. Subsequently it was determined that lauric acid was a fatty acid better suited as a marker of absorption since there are minimal amounts (0.8 gm/day) in the normal human diet (<http://www.barc.usda.gov/bhnrc/foodsurvey/home.htm>) and it does not undergo appreciable bacterial alteration.

Prior to our current study, we studied 4 adult control participants. In 2 participants, the 72 hour stool collections were obtained with the fecal behenate method and fat balance method concurrently performed. The fecal behenate method indicated absorption of 95.1 and 91.9% of dietary fat while the 72 hour fat balance method indicated absorption of 67.5 and 95.1%, respectively. In the other 2 participants, only the fecal behenate method was performed and fat absorption was calculated to be 99.9 and 100% (unpublished results). These results suggested that the fecal behenate method was quite robust in control participants and indicate that the method might be quite useful for differentiating normal vs. excessive fecal fat loss. Those studies encouraged us to pursue the current investigations in CF patients.

The current study was designed to test the hypothesis that the ratio of stool lauric:behenic acid would be predictive of the coefficient of fat absorption using the 72 hour fat balance method in children and adults with cystic fibrosis (CF) when they were not taking their customary pancreatic enzyme supplements and when taking enzyme supplements before or throughout the meal.

MATERIALS AND METHODS

Patient Population

Participants were recruited from the Cystic Fibrosis Clinic at Cincinnati Children's Hospital Medical Center (CCHMC) and The Children's Hospital (Aurora, CO) and the Pulmonary Clinic at the University of Cincinnati. Eligible participants were patients, age 7 to 50 years with CF confirmed by sweat electrolytes and/or genotype with pancreatic insufficiency treated with pancreatic enzyme replacement therapy (PERT) and stable or increasing weight for at least 3 months prior to recruitment. Participants were required to be on stable PERT for the preceding 3–6 months. Exclusion criteria included 1) Pregnancy; 2) The presence of other diseases causing fat malabsorption; 3) Need for total parenteral nutrition; 4) Any change in their usual antibiotic therapy in the month preceding the periods of stool collection. Participants

were approached by the lead investigator at each site (JD, MN), and assessed regarding inclusion/exclusion criteria. If they met entry criteria, a verbal and then written explanation of the study was provided to the participant. The study was approved by the Children's Hospital and University of Colorado Denver, as well as the CCHMC and University of Cincinnati Institutional Review Boards and the Scientific Advisory Boards of the General Clinical Research Center at CCHMC and The Children's Hospital.

Experimental Methods

The study design was a cross-over, whereby one group of participants was initially on PERT and then repeated the study off PERT, and the other group was initially off PERT and then on PERT. The independent variables analyzed were percent fat absorption assessed by the lauric acid/behenic acid and 72-hour fat balance method, both continuous variables. The primary dependent variable in this study is fat malabsorption status. Participants were randomized to 3 days without pancreatic enzyme supplements or their usual supplements administered before meals. It was anticipated that most participants would be taking PERT prior to meals and not throughout; however, this was noted specifically as it was relevant to the studies. Participants were encouraged to select a high-fat (at least 70g fat/day) diet, and a trained research dietitian (SS, Janine Higgins, or Melanie Kasten) analyzed their intake to calculate total energy and fat intake. The participants were admitted to the General Clinical Research Center at Children's Hospital Medical Center or the Clinical Translational Research Center at The Children's Hospital, Aurora, CO. Each adolescent/adult participant was fed a diet of their own choosing containing at least 70 grams of fat. This fat intake represents 30% of calories in a 2000-calorie diet. Children were also provided diets of their own choosing with proportional fat and calorie intake appropriate for age. Prior to admission, participants were given a color marker (Brilliant Blue) by mouth. Participants who were to be off of their PERT for the admission stopped taking their pancreatic enzymes three days prior to the ingestion of this marker. When the first marked stool was identified by the participant, the participant was admitted to the GCRC. Thereafter stools were collected individually and then pooled when the collection was complete. Seventy-two hours after the identification of the color marker in the stool, a second marker was administered and all stools collected including the 2nd marked stool. On the second day of the hospitalization, the first meal consisted of fat free pudding with sucrose polybehenate (SPB), a component of fat used in the commercial preparation of fried snack food (a gift from the Procter & Gamble Company, Cincinnati, OH) and coconut oil. Coconut oil was purchased from The Kroger Co. (Cincinnati, OH). These components were blended at a weight ratio of 95:5 coconut oil: SPB and mixed with fat-free chocolate or vanilla pudding. One serving contained 485 Kcals with 6.94 g protein, 46.04 g carbohydrate, and 30.91 g fat consisting of 3.38 g saturated fat, 6.34 g monosaturated fat and 19.83 g polyunsaturated fat. The test meal was served as breakfast and supplemented with other fat-free products such as fat-free beverage including skim milk, fruit juice, black coffee/tea and/or piece of fresh fruit. The participants did not eat for the next three hours and thereafter resumed their usual diet.

Approximately 100 mg of feces was removed from the next 2–4 stools passed after the test meal for lauric acid:behenic acid analysis by gas chromatography techniques; This volume of stool represents <0.1% of the total stool collected over 72 hours and had minimal impact on the results of the 72 hour collection. Results were expressed as the average of the ratio of lauric acid:behenic acid contained in 2–3 stools collected after meal consumption.

Fatty acid compositions of the pudding with added fat and of the feces were analyzed by gas chromatography using the method of Metcalfe et al.(3,4). The fraction of fat absorbed was calculated using the following equation:

$$\text{Fraction of absorbed fat} = \frac{\frac{F_d}{B_d} - \frac{F_f}{B_f}}{\frac{F_d}{B_d}} \times 100\%$$

Where F_d = mass of dietary lauric acid in the test meal

B_d = mass of dietary behenic acid

F_f = mass of fecal lauric acid

B_f = mass of fecal behenic acid

The fat content of all food consumed was calculated to obtain total fat intake for each participant. At the end of the 72-hour collection, all stools were pooled and analysis performed using nuclear magnetic resonance (5,6) by Mayo Clinic Laboratories (Rochester, MN). All results were expressed as the coefficient of fat absorption:

$$\text{Coefficient of fat absorption (CFA)\%} = \frac{\text{dietary fat intake} - \text{fecal fat output}}{\text{dietary fat intake}} \times 100\%$$

Subsequently, participants either used their standard pancreatic enzyme supplements or no supplements for 3 days as outlined above in preparation for the 2nd study period. Participants then repeated the study using their pancreatic enzyme supplements throughout the duration of the meal for the 3rd study period (enzymes would be administered equally at the beginning, middle, and end of the meal).

Statistical Considerations

a. Sample Size Calculation—The sample size was based on comparison of the methods when participants were either receiving or not receiving PERT. The expectation is that when the participants are on PERT, the amount of fat absorption would be 85%, as compared to 60% when they are not receiving supplements. Thus, the reduction in one group is 25% and in the other group it is –25%. The standard deviation is not known, so to be conservative a SD equal to 20% was used. Using the sample size procedure for a two-sample t-test, the n required was computed. The software used was nQuery®, version 5.0 for a two-sided t-test with a level of significance of 0.05 and a power of 80%. For this aim (Aim 1), 9 individuals crossed over would be sufficient. The assessment of differences between PERT during meals vs before meals was considered exploratory and therefore a pilot study (Aim 2). It was anticipated that the sample size selected for Aim 1 might not be sufficient to determine the impact of using PERT before vs. during the meal. The results of this preliminary study might be used to assess the potential size of a population needed to answer this question if future studies were undertaken. Using a crossover design, we projected that means of absorption may be 10% or –10% with a standard deviation of 20%. For this approach a sample size of 34, calculated using n Query®, version 5.0 for a paired t-test, would be required to be able to detect the difference of 10% between participants ingesting pancreatic enzymes before vs. during the course of the meal. Since it was not practical to perform the entire study of Aim 2, we compromised and initiated studies with 15 participants as a pilot that should inform us regarding a more accurate sample size for a definitive study.

b. Data Analysis—Prior to any analysis, SPSS version 16.0.1 was used to generate means, standard deviations, skewness, range, and other descriptive statistics. Analysis of variance was used to analyze the data from the crossover study (off PERT vs. on PERT and on PERT before

meals vs. PERT during meals) to determine difference in fat absorption calculated by the “gold standard” 72 hour quantitative fecal fat determination. Comparisons of fat absorption by the behenate method vs. the 72 hour fat balance method across all conditions were made using the Pearson Correlation coefficient.

RESULTS

Eighteen participants met inclusion criteria and were enrolled in the study. Fifteen participants completed at least 2 of the arms of the study. Fourteen participants completed all 3 arms and one did not complete the arm in which PERT was consumed throughout the meal. Participants were age 8–50 years with a mean age of 21.1 years and a median age of 19.5 years. There were 7 males and all were Caucasian. Participants were well nourished with a body mass index (BMI) in adults (greater than age 18 years) of 17.8–30.3 while for children the BMI ranged from the 10th to 75th percentile for age. Nine participants were receiving antacids (ranitidine or a proton pump inhibitor), 3 on ursodeoxycholic acid (all with mild CF-associated liver disease), 11 on supplemental vitamins, 4 were on stable antibiotic regimens, and 10 were taking medications for pulmonary disease. One patient had diabetes mellitus and was receiving insulin. The mean dose of PERT administered was estimated to be 1528 U/kg/meal.

There was no difference between fat absorption measurements assessed by the 72 hour fat balance method compared to the test method (fecal behenate method) while not receiving PERT (Figure 1). Fat absorption was similar ($p=ns$) when assessed by the fecal behenate method ($51.7 \pm 21.6\%$, mean $\pm$ S.D.) compared to the 72 hour fat balance method ($51.5 \pm 22.7\%$). There was, however, a significant difference ($p=0.03$) between the fat absorption results assessed using the fecal behenate method ($64.5 \pm 28.3\%$) vs. the 72 hour fat balance method ($80.4 \pm 18.4\%$) with individual results while on PERT (Figure 2).

There was a trend toward concurrence for the 2 methods (Figure 3). For all studies performed for the 3 arms of the study, there was a significant correlation between the fat absorption assessed by the 72 hour fat balance method compared to fecal behenate method ($r^2=0.219$, $p=0.001$). We assessed fat absorption during periods when participants were not on PERT compared to when they were taking PERT either before or during meals. For the fecal behenate method, fat absorption was $51.7 \pm 21.6\%$ without PERT compared to $64.5 \pm 28.3\%$ taking PERT before meals and $67.6 \pm 21.1\%$ during meals. When we compared fat absorption by the 72 hour fat balance method for all participants completing all 3 arms of the study, we found fat absorption of $51.5 \pm 22.7\%$ without PERT compared to $80.4 \pm 18.4\%$ with PERT before meals and $78.3 \pm 15.7\%$ during meals. There was no significant difference ($p=ns$) in fat absorption when PERT was administered before or during meal consumption (Figure 4) for either method.

DISCUSSION

The results of the current study showed that the fecal behenate method which measures fecal lauric acid/behenic acid ratio after a test meal containing lauric acid mixed with other fatty acids, correlates with the “gold standard”, the 72 hour quantitative fecal fat measurement. Unfortunately, the correlation is not sufficiently robust that we could confidently suggest that the behenate method could replace the 72 hour fat balance method for the management of pancreatic enzyme treatment of patients with cystic fibrosis. In our preliminary studies, fat absorption does not appear to be impacted by whether enzymes are taken preceding or during the meal in cystic fibrosis. To definitively answer this question an extremely large sample ($n=329$ with 80% power and alpha of 0.05) would have to be studied to definitively indicate that there are differences in absorption resulting from the timing of enzyme administration.

The goal of this study was to determine if we could find a facile way to estimate fat absorption in patients with cystic fibrosis that could be used to monitor responses to interventions directed at correcting fat absorption. Given the complications that can result from fat malabsorption, accurate and timely identification is critical in the diagnosis and management of these patients. Although other tests of fat malabsorption have been proposed, these tests are limited in application, accuracy and feasibility. Several other diagnostic tests to identify fat malabsorption have been proposed. In 1961, Drummey et al. reintroduced the technique of microscopic examination of stool for fat globules and outlined a scale for grading this steatorrhea (7). Although the sensitivity of this method has been reported as high as 96% (8), these results have not been reproducible (9,10). Additionally, this test is only a semiquantitative measure with relatively poor specificity (11), thus patients with fat malabsorption identified by this method still need a 72-hour fat balance study to quantify and confirm this steatorrhea. Another method, the triolein breath test, measures exhaled $^{14}\text{CO}_2$ or $^{13}\text{CO}_2$ after ingestion of a known amount of triglyceride labeled with ^{14}C or ^{13}C . Reports have been mixed on the sensitivity and specificity of this test, with sensitivity varying from 64%–100% (12,13). Unfortunately, several other factors other than triolein absorption affect the rate of conversion to carbon dioxide and this test may be inaccurate in many diseases that lead to fat malabsorption such as cholestatic liver disease and cystic fibrosis (12,13). Due to these limitations, the triolein breath test is not commercially available for clinical use. Finally, in 1981, Phuapradit et al. introduced the steatocrit, a new screening method to estimate fecal fat that required only a small volume of stool, making it ideal for use in the pediatric population (14). This procedure was modified by Tran et al., who found that acidification of the stool prior to performing the steatocrit improved the fat separation and increased the sensitivity of this method (15,16). Unfortunately, the two initial studies by Tran et al. using this method were quite small and larger studies using the acid steatocrit have not been as promising, with a lower sensitivity (17) and a weak correlation between the acid steatocrit and the 72 hour fat-balance method (18). Recently, work using dysprosium chloride as a nonabsorbable marker with stably-labeled triglycerides as a method to assess fat absorption has been reported (19). This work has demonstrated that a single marked stool with brilliant blue in which the fractional excretion of a non-absorbable marker, dysprosium, is assessed can be compared to the presence of a labeled fatty acid. A high correlation was demonstrated between the fractional excretion of Dy and ^{13}C in stool indicating that this was a promising method to assess fat absorption on a single marked stool. Unfortunately, the measurement of Dy and ^{13}C require mass spectrometry, which is not routinely available, and material costs are expensive.

There are little data examining the effects of timing of pancreatic enzyme supplementation in relationship to meals. There is anecdotal evidence that administering enzyme supplements during the meal rather than before the meal may reduce gastrointestinal symptoms and potentially enhance fat absorption (personal communication, Preston Campbell). There has been no controlled study examining this question. Our results do not suggest a relationship between timing of PERT administration. Based upon the small differences observed in fat absorption in relationship to timing of PERT administration, the calculated large sample size needed to assess this question makes the performance of such a study in the future unlikely.

The reason for the suboptimal correlation between the fecal behenate method and the 72 hour fat balance method are not clear. There are a number of possibilities: 1) Absorption from a single meal, on which the fecal behenate method is based, may not be representative of the average fat absorption over the 3 day period over which the 72 hour fat balance method is based. There are little data on meal-to-meal and day-to-day variation in fat excretion in normal or diseased humans even though assays for fecal fat have been reported for over a century (20). Early work on fat absorption does suggest there is day-to-day variation in fat excretion in adults with jaundice reported secondary to cirrhosis or hepatitis (21). The results of additional studies that we have performed examining the effect of Xenical on fat absorption in 10

participants comparing the fecal behenate method with the 72 hour fat balance method using NMR analysis had correlations between 0.51 and 0.80 (unpublished results, JEH, RJ). 2) There may be intrinsic variability in fat absorption in CF as suggested by the failure to show robust correlations between the ^{13}C - triolein breath test and the 72 hour fat balance method (22); however, studies by Jongorbani et al suggest that the technique which utilizes stable-labelled triglyceride with a non-absorbable marker, dysprosium given as a single meal has a strong correlation with the 72 hour fat balance method in CF patients (18). 3) Although the 72 hour fecal fat method is considered the “gold standard” for assessing fat absorption, there is considerable variability of results in normal subjects and those with CF. In normals, the variability on test-retest can be -8.1 to $+5.9\%$ while in CF it may be larger with test-retest values of -19.7% to 42.8% (23) Additional unpublished results indicate large test-retest variability in CF patients on PERT (-30% to $+58\%$) or on placebo ($+42$ to -42%) (personal communication, D. Borowitz). Additional factors may play a role although likely to be minor. Conditions causing interruption of the enterohepatic circulation of bile acids, such as ileal resection, lead to intraluminal bile acid concentrations falling below the critical micellar concentration as the day progresses (24). Although patients with CF have mild perturbations in the enterohepatic circulation and none of our participants had a previous ileal resection, it seems unlikely that they have sufficient reductions in intraluminal bile acids with meals during the day to explain the discrepancies (25–27). 3) It is possible that dietary fat containing lauric acid might have compromised the results since the fecal behenate method is dependent upon knowing the dietary lauric at and around the time of the test meal. This seems unlikely since lauric acid is contained in only a limited number of foods and the participants diets were carefully controlled in a hospital setting. 4) Half of the subjects were on some type of acid suppression therapy. This might have had some impact on their absorption of dietary fats especially if there was any variability in their compliance with the medication. 5) Additional factors in CF may have some impact on fat absorption that have been incompletely explored including bacterial overgrowth, variability in release of exogenous pancreatic enzymes during the course of the day, and recently recognized mucosal abnormalities in absorption.

Despite the somewhat discouraging results of the present study, there are some encouraging findings. The behenate test does correlate with fat absorption and, with modification, may ultimately prove useful in this regard because of its ease of sample collection and analytical requirements. Investigators should be encouraged to continue to pursue development of facile methods for assessing fat malabsorption that may be used in adult and children with diarrhea for diagnosis and management of their diseases.

Acknowledgments

The authors acknowledge the contributions of the research participants and their families, Churee Pardee, B.S., R.N. and the staff of the GCRCs at Cincinnati Children’s Hospital Medical Center and the Denver Children’s Hospital, Procter and Gamble for the gift of sucrose polybehenate, and support from Cystic Fibrosis Foundation and the General Clinical Research Center RR008086 and RR00069.

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Figure 1. Relationship between % fat absorption by the balance method and the fecal behenate method in CF patients without PERT

Figure 2.
Relationship between %fat absorption by the balance method and the fecal behenate method
in CF patients with PERT

Figure 3. Correlation between fat balance method and fecal behenate method for in CF patients without PERT, with PERT before meals and with PERT during meals

Figure 4. Relationship between % fat absorption by the balance method in CF patients without PERT, with PERT before meals and with PERT during meals