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Malabsorption Blood Test: Assessing Fat Absorption in Patients With Cystic Fibrosis and Pancreatic Insufficiency

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Abstract

The malabsorption blood test (MBT), consisting of pentadecanoic acid (PA), a free fatty acid, and triheptadecanoic acid (THA), a triglyceride that requires pancreatic lipase for absorption of the heptadecanoic acid (HA), was developed to assess fat malabsorption in patients with cystic fibrosis (CF) and pancreatic insufficiency (PI). The objective was to construct a population pharmacokinetic (PK) model to describe PA and HA disposition in healthy subjects and CF subjects. A model was simultaneously fit to PA and HA concentrations, consisting of 1-compartment disposition and a transit model to describe absorption. PA bioavailability estimates for CF subjects without pancreatic enzyme administration (1.07 [0.827, 1.42]) and with enzymes (0.88 [0.72, 1.09]) indicated PA absorption comparable to healthy subjects. HA bioavailability in CF without enzyme administration was 0.0292 (0.0192, 0.0459) and with enzymes increased to 0.606 (0.482, 0.823). In CF, compared with taking enzymes with the MBT, HA bioavailability was further decreased by factors of 0.829 (0.664, 0.979) and 0.78 (0.491, 1.13) with enzymes taken 30 and 60 minutes after MBT, respectively. The MBT detected differences in fat absorption in subjects with CF with and without enzyme administration and with changes in enzyme timing. Future studies will address application of the MBT in CF and other malabsorption diagnoses.

Keywords

cystic fibrosis, fat malabsorption, pancreatic function test, pancreatic enzyme replacement therapy

Pancreatic insufficiency (PI) is seen in the majority of patients with cystic fibrosis (CF), resulting in maldigestion and malabsorption of fat, carbohydrate, and protein.[1] Patients are treated with pancreatic enzymes to improve nutrient absorption, growth, and nutritional status, although malabsorption often persists.[2] The accurate assessment of fat malabsorption is an important component of clinical care, especially in those with poor nutritional status.

Assessing the degree of fat malabsorption is helpful for guiding both enzyme therapy and nutritional intervention in patients with CF and PI. Seventy-two-hour stool and diet collection for coefficient of fat absorption (CFA) assessment is considered the standard test for measuring the degree of fat malabsorption,[3,4] and CFA is widely accepted as a reliable, but nonspecific index for assessing fat malabsorption. It does not differentiate among liver, pancreatic, and intestinal causes of fat malabsorption. The CFA has many limitations.[5] The technical and aesthetic difficulties associated with stool collection, storage, and analysis make this test unappealing to patients, families, and laboratory staff. Stool must be shipped to specialized laboratories for analysis. The accuracy of this test is dependent on 3 days of entire stool collections together with consumption and documentation of a moderate- to high-fat diet. Errors in fecal fat estimation occur because of inadequate documentation of dietary intake, incomplete stool collections, and day-to-day variation in fecal fat excretion and number of stools passed.[6] With these limitations, CFA is underutilized or avoided in most clinical care and research settings. Several alternative tests for assessment of fat malabsorption have been explored including mixed triglyceride, dipeptide breath tests,[7] stable and radioactive isotope tests, spot stool fat level,[8] stool behenate levels,[9] secretin-cholecystokinin–stimulated pancreatic challenge test, and secretin-stimulated magnetic

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resonance cholangiopancreatography.[10–12] All have limitations and have failed to gain wide acceptance as accurate or practical alternatives to the 72-hour stool and diet collection. The most accepted alternative test, which at this time is a research method, is the ^{13}C-mixed triglyceride test,[13–15] and is not available in the United States. Comparing 9 children with CF and PI with 10 healthy controls, Herzog et al[16] noted that this test was able to distinguish normal pancreatic function from PI but with wide within-subject variability. The requirement of a radioactive label limits its use in pediatric patients.

The malabsorption blood test (MBT) was developed in response to the need for a more accurate, acceptable alternative method to the CFA and the other alternative tests available to assess pancreatic-based fat malabsorption in CF. Furthermore, although pancreatic enzyme administration is a successful component of nutritional intervention in CF, the optimal timing of the administration of enzymes has been derived empirically from clinical experience, with little evidence of an enzyme time-response relationship to support clinical management. The MBT may provide an evidence-based method for guiding enzyme administration and a clearer understanding of the pancreatic enzyme and malabsorption relationship.

The MBT uses 2 naturally occurring fatty acids with odd-number carbon atom chain length: pentadecanoic fatty acid (PA) and triheptadecanoic acid (THA). PA, a free fatty acid, is absorbed without the need for pancreatic lipase, whereas THA, a triglyceride, requires hydrolysis by lipase to heptadecanoic acid (HA) before absorption. The principle behind the MBT is that the postdose difference in serum concentrations of the 2 fats (PA and HA) reflects the degree of pancreatic-based fat absorption.[17] The MBT has been shown to detect fat malabsorption in a group of healthy subjects on a fat absorption-blocking medication and in subjects with CF with and without the administration of pancreatic enzymes.[17]

The aim of this study was to construct a population model to describe HA and PA pharmacokinetics (PK) after MBT administration. This model will add to the previous MBT proof-of-concept work, and describe: (1) PA and HA disposition in a healthy comparison group of subjects, (2) PA and HA disposition in subjects with CF (both with and without enzyme administration), (3) the sensitivity of the MBT to changes in the timing of enzyme administration, and (4) between-occasion variability in HA and PA exposure. This will provide additional evidence that the MBT may be an acceptable alternative test to the CFA.

Methods

Subjects

The study protocols were approved by the Committee for the Protection of Human Subjects (institutional review board) at the Children's Hospital of Philadelphia (CHOP). A parent or guardian provided consent for children younger than 18 years. Informed consent was obtained for adult subjects ($\geq$18 years of age) and assent from children ages 8–17 years. Subjects with CF and PI were recruited from CHOP and the Pennsylvania Presbyterian Medical Center. Healthy young adult volunteers were recruited from the community. Inclusion criteria for the subjects with CF included $\geq$8 years of age, PI confirmed by a fecal elastase 1 of $<$200 ug/g stool, and usual state of good health. Exclusion criteria included FEV_1 % predicted of $<$40%, history of fibrosing colonopathy, significant bowel resection ($>$10 cm) or endocrine or gastrointestinal disorders. Exclusion criteria for the healthy subjects included: any chronic illness known to affect nutrient absorption, body mass index $<$21 or $>$30 kg/m^2, lipid-lowering drugs, and endocrine or gastrointestinal disorders.

Subjects who participated in several protocols were combined into the CF and healthy comparison groups for this study.

Subjects with CF (n = 33) participated in the following 3 protocols:

1. No Enzymes Protocol: Subjects with CF (n = 6) participated in a protocol with 2 MBTs, one without enzyme administration at the time of the MBT and one with enzyme administration typical for a dinner meal with the MBT.[17]
2. Timing of Enzymes Protocol: Subjects with CF (n = 16) underwent the MBT on 4 separate occasions, each at least 5 days apart. A standard dose of enzymes was administered randomly at 1 of 4 times: (1) 30 minutes premeal, (2) immediately at the initiation of the meal, (3) 30 minutes postmeal, and (4) 60 minutes postmeal. When it was noted in the interim data review that PA and HA concentrations were notably reduced when enzymes were given 60 minutes postmeal, this arm of the study was discontinued after 9 subjects to reduce subject burden.
3. Reproducibility Protocol: Subjects with CF (n = 11) underwent the MBT on 3 separate occasions at least 5 days apart.

Healthy subjects for the comparison group (n = 27) participated in 2 protocols:

1. Orlistat Protocol: The healthy subjects (n = 15) participated in our Orlistat Protocol described previously,[17] and for this analysis, the MBT prior to Orlistat administration was used.

2. Timing of Enzymes Protocol: Healthy subjects (n = 12) served as a comparison group for the Timing of Enzymes Protocol in subjects with CF (see above). They underwent the MBT as part of a study to determine gastric and small bowel meal transit as described in Rovner et al.[18]

Study Protocol

The study was conducted in the Clinical and Translational Research Center in the early morning after a 12-hour fast. Participants abstained from alcohol or dairy products for 24 hours prior, but otherwise consumed their typical diet. This was confirmed by a 24-hour dietary recall the day of the MBT. Subjects maintained a typical diet and usual physical activity for 48 hours prior to MBT. Weight was measured on a digital scale (Scaletronix, White Plains, New York) to 0.1 kg and height measured on a stadiometer (Holtain, Crymych, UK) to 0.1 cm.

MBT Preparation

Participants consumed the MBT 8-ounce test meal, which contained 550 calories, 32 g of fat, Chocolate Scandishake powder (http://www.nutricia.com), vanilla low-fat soy-milk, microlipids (http://www.nestle-nutrition.com), 2.5 or 5.0 g of PA (15-carbon saturated fatty acids, C15:0), and 5.0, 5.5., or 8.0 g of THA (a triglyceride with 3 heptadecanoic acids (HA; 17-carbon saturated fatty acids, C17:0). Note that the amount of study fats varied across different protocols, as the fat dose was optimized for the MBT. Healthy subjects in the Orlistat Protocol (n = 15) ingested 2.5 g of PA and 8.0 g of THA, whereas those in the Timing of Enzymes Protocol (n = 12) ingested 5.0 and 5.5 g, respectively. Subjects with CF ingested 2.5 g of PA and either 5.0 g (n = 3) or 8.0 g (n = 3) of THA in the No Enzymes Protocol, and 5.0 g PA and 5.5 g THA in the Timing and Reproducibility Protocols (n = 27); see Table 1. Study participants ingested the liquid study meal within 5 minutes. Plasma samples were obtained at hourly intervals over 8 hours beginning with the baseline premeal sample. During the test, all subjects were permitted ad libitum noncaloric and noncaffeinated beverages. After the 6-hour blood draw, subjects received a 1000-kcal, low-fat (12-g) lunch meal. Subjects with CF ingested a standard dose of enzymes (4 capsules of Creon 20 [http://www.creon.com], 80 000 lipase units) with the MBT and also with lunch. If a subject typically took a higher dose of enzymes for a lunch/dinner meal, the equivalent higher dose of Creon 20 was provided. The number of Creon 20 capsules ingested with the MBT and with the lunch meal varied from 4 to 7 (80 000–140 000 pancreatic lipase units): 52% took 4 (80 000 units), 15% took 5 (100 000 units), 21% took 6 (120 000 units), and 12% took 7 (140 000 units) Creon 20 capsules.

Sample Analysis

Plasma samples were analyzed using a standardized gas chromatographic method. The details have been previously published.[17] Interassay variability (%CV) for the measurement of PA in samples with low, medium, and high concentrations (1.30, 2.99, and 6.70 mg/dL) was 2.9%, 2.6%, and 3.1%, respectively. Interassay variability for the measurement of HA (0.56, 1.29, and 3.05 mg/dL) in the same samples was 2.6%, 4.0%, and 3.9%, respectively.

Table 1. Characteristics of Subjects Participating in MBT Studies by Protocol

Sample	N	Sex, %	Age, y	Weight, kg	PA dose, g	THA dose, g
HEALTHY						
Orlistat Protocol	15	60	31.5 ± 8.5 29.5 [21.5–49.9]	78.6 ± 11.5 77.9 [60.0–95.4]	2.5	8.0
Timing Protocol	12	50	28.6 ± 9.2 27.0 [19.0–47.0]	74.6 ± 14.3 68.0 [61.4–101.6]	5.0	5.5
TOTAL HEALTHY	27	56	33.2 ± 8.7 28.2 [19.0–49.9]	76.8 ± 12.7 71.6 [60.0–101.6]		
CYSTIC FIBROSIS						
No Enzymes	6	50	15.4 ± 2.6	48.6 ± 5.5	2.5	5.0 (n=3)
Protocol			14.4 [13.3–19.9]	49.6 [40.6–55.6]	2.5	8.0 (n=3)
Timing Protocol	16	44	16.4 ± 3.6 16.8 [10.9–23.5]	49.6 ± 10.6 53.7 [30.7–64.8]	5.0	5.5
Reproducibility Protocol	11	45	16.8 ± 4.4 15.6 [9.9–24.2]	50.5 ± 12.5 49.4 [34.8–74.5]	5.0	5.5
TOTAL CF	33	45	16.4 ± 3.7 15.6 [9.9–24.2]	49.7 ± 10.3 50.5 [30.7–74.5]		

Mean ± SD
Median [Range]

Population Pharmacokinetic (PK) Analysis. Population PK analyses for repeated-measures end points were conducted via nonlinear mixed-effects modeling with qualified installation of the nonlinear mixed-effects modeling (NONMEM) software, Version VII, Level 2.0 (ICON Development Solutions, Hanover, Maryland). The first-order conditional estimation method with η-ϵ interaction (FOCE-INT) was employed for all model runs.[19] Population PK modeling was conducted by simultaneously fitting structural PK models to both PA and HA concentrations. Initial models used 1-compartment models for PA and HA with first-order absorption parameterized in terms of apparent clearance (CL/F), apparent volume of distribution (V/F), and absorption rate constant (K_a), with random effect distributions for all parameters. Fasting baseline PA (PA_0) and HA (HA_0) concentrations were also estimated to account for variability in concentration of fats at baseline prior to MBT administration. An exponential variance model was used to describe the variability of PK parameters across individuals in the form:

$$P_i = \hat{P}\exp(\eta_{Pi})$$

where P_i is the estimated parameter value for individual i, $\hat{P}$ is the typical population value (geometric mean) of the parameter, and η_{Pi} is individual-specific interindividual random effects for individual i and parameter P and are assumed to be distributed: $\eta \sim N(0, \omega^2)$, with covariances defined by the interindividual covariance matrix Ω. A full covariance matrix was estimated to capture random-effects correlation in both PA and HA models.

Residual error was described by the proportional error model:

$$Y_{ij} = \hat{Y}_{ij}(1 + \epsilon_{pij})$$

where Y_{ij} is the *jth*-measured observation (plasma PA or HA concentration) in individual i, $\hat{Y}_{ij}$ is the *jth* model predicted value (plasma PA or HA concentration) in individual i, and ϵ_{pij} is the proportional residual random for individual i and measurement j and is assumed to be independently and identically distributed: $\epsilon \sim \text{NID}(0, \sigma^2)$.

Covariate Modeling

A covariate modeling approach emphasizing parameter estimation rather than stepwise hypothesis testing was implemented for this analysis. First, covariate–parameter relationships were identified based on scientific interest, prior knowledge, and physiologic plausibility. A covariate model was carefully constructed to avoid correlation or collinearity in predictors. Population parameters, including fixed-effects parameters (covariate coefficients and structural model parameters), and random-effects parameters were estimated. Inferences about relevance of parameters were based on the resulting parameter estimates of the full model and measures of estimation precision. For this analysis, body weight, subject status (healthy versus CF groups), administration of pancreatic enzymes, and pancreatic enzyme timing represented the covariates of interest.

Model Evaluation

The precision of model parameters was investigated by performing a stratified nonparametric bootstrap procedure.[20] Five hundred replicate data sets were generated by random sampling with replacement and stratified by subject status (CF vs healthy groups) and enzyme administration method, using the individual as the sampling unit. Population parameters for each data set were subsequently estimated for each replicate data set and empirical 95% confidence intervals (CIs) were constructed by observing the 2.5th and 97.5th quantiles of the resulting parameter distributions for those bootstrap runs with successful convergence.

The performance of the final model was also investigated via a visual predictive check to determine if the model accurately reproduced the variability in the observed data.[21] Five hundred Monte Carlo simulation replicates of the original data set were generated using the final population PK model. Plots of the observed data were constructed, overlaid with the simulated median and 5th and 95th percentiles for comparison with the observed data distribution.

Results

Subject Demographics

Subject characteristics are summarized in Table 1. Subjects with CF were somewhat younger than the healthy subjects (15 ± 3 and 32 ± 9 years, respectively) and had lower body weights (49 ± 6 and 79 ± 12 kg, respectively). By design, the healthy comparison group consisted of young adults due to the Orlistat Protocol. Doses of PA and THA for each protocol group of both healthy comparison subjects and subjects with CF are provided in Table 1. Subjects with CF participated in various protocol schedules, 2 MBTs, one with and one without administration of pancreatic enzymes ($n = 6$), repeated MBTs on 3 different occasions ($n = 11$) in the Reproducibility Protocol, or MBT repeated with variations in enzyme timing on 3 or 4 occasions within the same subject ($n = 16$). For the healthy comparison group, 1 MBT was used in these analyses, the MBT prior to Orlistat administration in the Orlistat protocol ($n = 15$) or the MBT given as part of the Timing of Enzymes Protocol ($n = 12$).

For subjects with CF, the Protocols required a minimum 80 000 lipase unit dose of pancreatic enzymes unless the participant typically took a higher dose: 52%

took 80 000 lipase units with the MBT, and higher doses for other subjects were 100 000–140 000 lipase units.

Population Pharmacokinetic Analysis. Initial model fits for a 1-compartment model with first-order absorption and a baseline concentration were biased and did not appropriately describe PA and HA absorption. Various absorption models were fitted with the 1-compartment disposition model during the building process, including adding lag times, zero-order, sequential zero, and first-order, and transit compartment models. The transit model[22] best described PA and HA absorption.

The change in apparent clearance (CL/F) and volume of distribution (V/F) as a function of body size was described by an allometric model,[23] where the typical value of a model parameter was described as a function of individual body weight (WT_i), normalized by a reference weight, which was 70 kg. Separate bioavailability fractions (relative to healthy subjects) were estimated for subjects with CF both with and without enzyme administration. A separate categorical effect was also included to describe changes in HA bioavailability in response to changes in enzyme timing.

PA and HA random-effects parameters were highly correlated for CL/F (0.974), V/F (0.987), and baseline concentrations (0.999). Therefore, random-effects parameters for HA were modeled as a fraction of the corresponding PA random effects. Random effects for between-occasion variability in absorption mean transit time and bioavailability for CF subjects with administration of enzymes were also included to describe variability in PA and HA exposures after repeat administration.

The final model was represented as:

PA Model	HA Model
$CL/F = \theta_1 \cdot \left(\frac{WT_i}{70\,kg}\right)^{0.75} \cdot e^{\eta_1}$	$CL/F = \theta_9 \cdot \left(\frac{WT_i}{70\,kg}\right)^{0.75} \cdot e^{(\eta_1 \cdot \theta_{20})}$
$V/F = \theta_2 \cdot \left(\frac{WT_i}{70\,kg}\right)^{1.0} \cdot e^{\eta_2}$	$V/F = \theta_{10} \cdot \left(\frac{WT_i}{70\,kg}\right)^{1.0} \cdot e^{(\eta_2 \cdot \theta_{21})}$
$PA_0 = \theta_3 \cdot e^{\eta_3}$	$HA_0 = \theta_{11} \cdot e^{(\eta_3 \cdot \theta_{22})}$
$MTT = \theta_4 \cdot e^{\eta_{PA,JOV,MTT}}$	$MTT = \theta_{12} \cdot e^{\eta_{PA,JOV,MTT}}$
$K_a = \theta_5$	$K_a = \theta_{13}$
$N = \theta_6$	$N = \theta_{14}$
$F_{CF} = \theta_7$	$F_{CF} = \theta_{15}$
$F_{CF+Enz} = \theta_8 \cdot e^{\eta_{PA,JOV,F}}$	$F_{CF+Enz} = \theta_{16} \cdot \theta_{17}^{t1} \cdot \theta_{18}^{t2} \cdot \theta_{19}^{t3} \cdot e^{\eta_{HA,JOV,F}}$

where CL/F is the apparent clearance; V/F is the apparent volume of distribution, PA_0 and HA_0 are the baseline concentrations; MTT is the absorption mean transit time; K_a is the first-order absorption rate; N is the number of transit compartments; F_{CF} is the relative bioavailability for CF subjects without enzyme administration; F_{CF+ENZ} is the bioavailability for CF subjects administered enzymes; θ_{17}, θ_{18}, and θ_{19} are the effect estimates for enzyme administration at -0.5 (30 minutes prior to MBT), 0.5 (30 minutes after MBT), and 1 hour after MBT; t1, t2, and t3 are indicator variables for enzyme timing; and θ_{20}, θ_{21}, and θ_{22} are factors for shared PA and HA random effects.

Diagnostic plots revealed that the model was consistent with the observed data, and no systemic bias remained (Figure 1). Parameter estimates for the full model are shown for PA and HA in Table 2. Along with the parameter point estimates, measures of parameter estimation uncertainty were also obtained. These included asymptotic standard errors, obtained from the NONMEM $COVARIANCE step, and 95%CIs, determined by nonparametric bootstrap. Overall, structural model parameters and covariate effects were estimated with reasonable precision, with the exception of HA V/F and the number of transit compartments for PA.

The PA bioavailability estimate of 1.07 (95%CI, 0.827, 1.42) for subjects with CF was similar to healthy subjects. The estimate for CF subjects administered enzymes was 0.88 (0.72, 1.09). Although 12.3% lower than in healthy subjects, the 95%CI contains the null value of 1. These results indicate that PA absorption is similar for healthy subjects and subjects with CF. The HA bioavailability for CF subjects without administration of pancreatic enzymes was 0.0292 (0.0192, 0.0459), suggesting that there is very little HA absorption in CF when enzymes are not administered. With enzymes, bioavailability increased to 0.606 (0.482, 0.823).

HA bioavailability was slightly decreased by a factor of 0.911 (0.710, 1.12) when enzyme administration occurred half an hour prior to the MBT. Given the small decrease and 95%CI that included the null value of 1, enzyme administration 30 minutes prior to the MBT yielded similar exposure to simultaneous administration. When enzymes were taken at 30 and 60 minutes following the MBT, HA bioavailability was decreased by factors of 0.829 (0.664, 0.979) and 0.78 (0.491, 1.13), respectively. Although the 95%CI for the 1-hour group contained the null value, the CI had a relatively wide range. This imprecision is likely a result of the reduced number of subjects that made up this group (n = 9).

PA and HA absorption was moderately variable within subjects. For mean absorption transit time (MTT), between-occasion variability was estimated to be 45.3% and 31.9%, and for bioavailability it was 30.4% and 36.9% for PA and HA, respectively.

To explore potential effects of age in this analysis, we conducted a post hoc covariate analysis by adding age effect models on PA and HA CL/F to the full covariate model. The age effect was estimated as:

$$CL * (AGE/18)\hat{\theta}(AGE)$$

where θ(AGE) is an estimated exponent to describe the age effect on CL/F. Adding these effect models resulted in no drop in the objective function value when compared

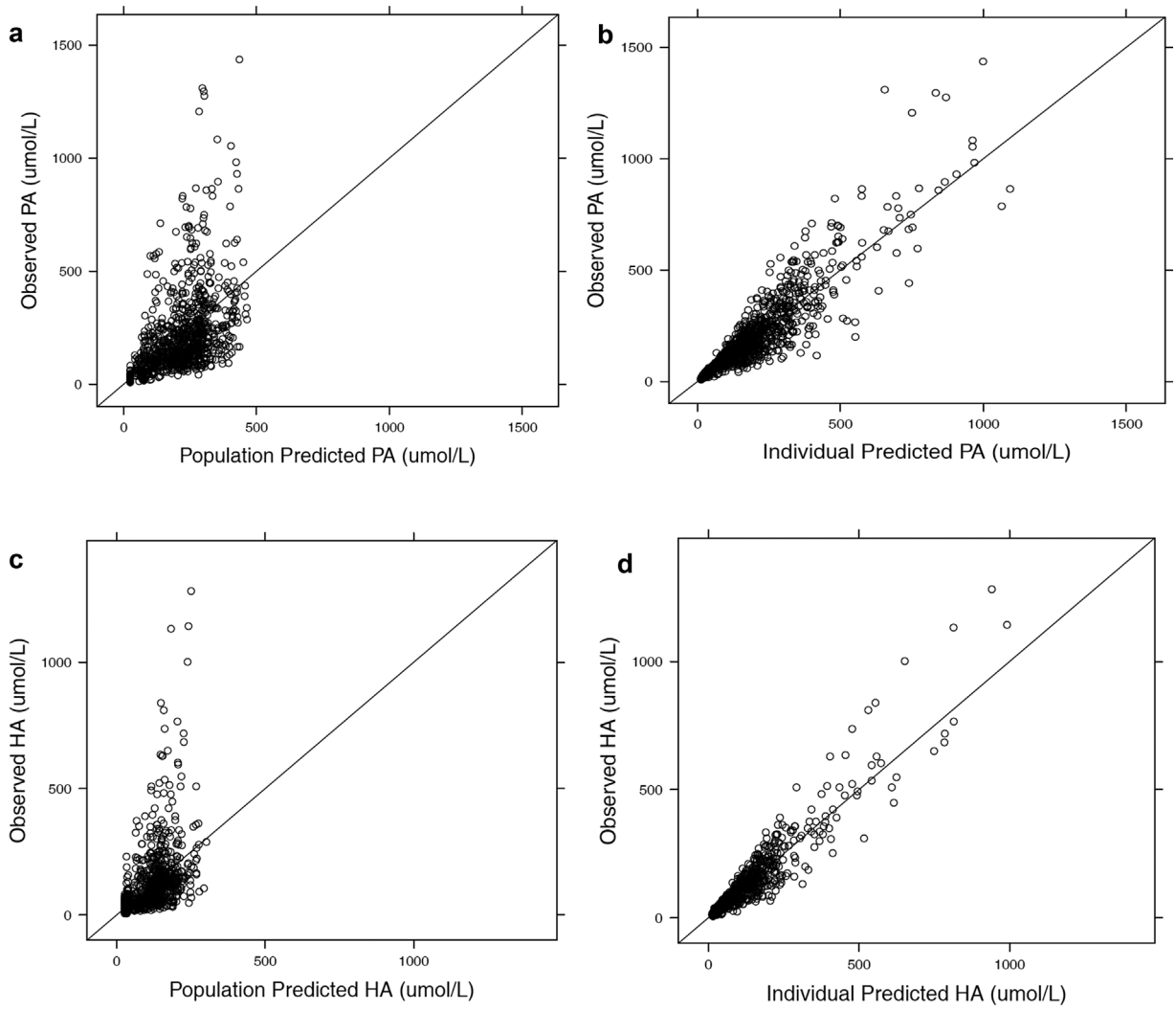

Figure 1. Diagnostic plots from the final population pharmacokinetics model. (a) PA observed versus population predicted. (b) PA observed versus individual predicted. (c) HA observed versus population predicted. (d) HA observed versus individual predicted.

with the reported model. Estimated exponents (95%CI) for the age effect were -0.421 (-1.11, 0.267) for PA CL/F and -0.995, 0.467 (-0.995, 0.467) for HA CL/F. Although the 95%CIs include the null value of zero, the low precision of the estimates indicates insufficient information in the data set to accurately characterize any age effects on CL/F. The dose of Creon 20 given with the MBT did not affect the results. In post hoc population PK analysis of the current model, Creon 20 doses of 100 000, 120 000 and 140 000 lipase units did not yield an increase in bioavailability when compared with the standard dose of 80 000 lipase units (not shown).

Model Evaluation

The visual predictive check revealed that the full model provided a reliable description of the data with good precision of structural model and variance parameter estimates. Figures 2, 3, and 4 show the observed PA and HA concentrations versus time for healthy subjects, subjects with CF, and subjects with CF with varying enzyme timing. Overall, simulated distributions were similar to the observed PA and HA concentrations.

Discussion

Based on the results of our study, and adding to the previous proof of concept findings, the MBT has promise as an alternative to the CFA requiring a 72-hour stool collection and diet record. The objectives of this population analysis were to characterize the PK of PA and HA after administration of the MBT in subjects with CF and a healthy comparison group. By including subjects from multiple protocols and expanding the sample size for both the CF and healthy comparison groups in the present study, modeling of the PA and HA pharmacokinetics was refined and expanded to include

Table 2. PA and HA Parameter Estimates From the Final Population Pharmacokinetic Model

			PA					HA				
Parameter	Description	Units	Estimate	RSE	95%CI	BSV	BOV	Estimate	RSE	95%CI	BSV	BOV
CL/F	Clearance	L/h	9.66	18.5%	(8.14, 12.4)	56.1%		16.3	15.8%	(13.9, 20.7)		
V/F	Volume of distribution	L	24.5	27.6%	(4.89, 32.5)	58.4%		14.3	64.1%	(1.35, 37.2)		
C_0	Baseline concentration	μmol/L	24.9	12.1%	(22.7, 27.4)	37.8%		27.4	9.5%	(25.3, 29.7)		
MTT	Mean transit time	h	0.817	7.3%	(0.751, 2.84)		45.3%	3.52	12.2%	(3.21, 4.33)		31.9%
K_a	First-order absorption rate	h^{-1}	0.266	17.6%	(0.206, 0.440)			0.307	17.2%	(0.253, 0.563)		
N	Number of transit compartments		6.96	39.1%	(0.195, 18.2)			7.08	20.6%	(5.79, 8.49)		
F_{CF}	Bioavailability — CF subjects		1.07	26.0%	(0.827, 1.42)		30.4%	0.0292	67.8%	(0.0192, 0.0459)		36.9%
$F_{CF,ENZ}$	Bioavailability — CF subjects with enzymes		0.877	30.0%	(0.720, 1.09)			0.606	26.9%	(0.482, 0.823)		
F_{T1}	bioavailability effect — enzymes at −0.5 h							0.911	21.0%	(0.710, 1.12)		
F_{T2}	Bioavailability effect — enzymes at 0.5 h							0.829	25.0%	(0.664, 0.979)		
F_{T3}	Bioavailability effect — enzymes at 1 h							0.78	26.7%	(0.491, 1.13)		
θ_{20}	Fractional change in HA CL/F random effect relative to PA							0.994	9.6%	(0.888, 1.17)		
θ_{21}	Fractional change in HA V/F random effect relative to PA							1.3	65.5%	(0.870, 2.43)		
θ_{22}	Fractional change in HA C_0 random effect relative to PA							0.998	19.9%	(0.890, 1.10)		
Interindividual variance												
Ω^2_{CL}	Interindividual variance of clearance		0.315	47.6%	(0.188, 0.416)							
$\Omega^2_{CL\text{-}V}$	Covariance for clearance and volume		0.162	51.7%	(−0.0666, 0.241)							
Ω^2_{V}	Interindividual variance of volume		0.341	55.4%	(0.230, 0.991)							
$\Omega^2_{CL\text{-}S0}$	Covariance for clearance and baseline		−0.0894	−51.7%	(−0.162, −0.0259)							
$\Omega^2_{V\text{-}S0}$	Covariance for volume and baseline		−0.151	−53.8%	(−0.231, −0.0899)							
Ω^2_{S0}	Interindividual variance of baseline		0.143	44.9%	(0.0892, 0.192)							
Intraindividual variance												
Ω^2_{F}	Intraindividual variance of bioavailability		0.0925	45.4%	(0.0565, 0.127)			0.136	44.8%	(0.0411, 0.189)		
Ω^2_{MTT}	Intraindividual variance of bioavailability		0.205	37.3%	(0.0471, 0.307)			0.102	37.3%	(0.0725, 0.127)		
Residual variance												
δ^2_{prop}	Residual proportional variance		0.0984	12.0%	(0.0877, 0.118)	31.4%		0.0774	10.1%	(0.0674, 0.0887)	27.8%	

SE, standard error of parameter estimate; RSE, relative standard error of parameter estimate; BSV, between-subject variability; BOV, between-occasion variability.

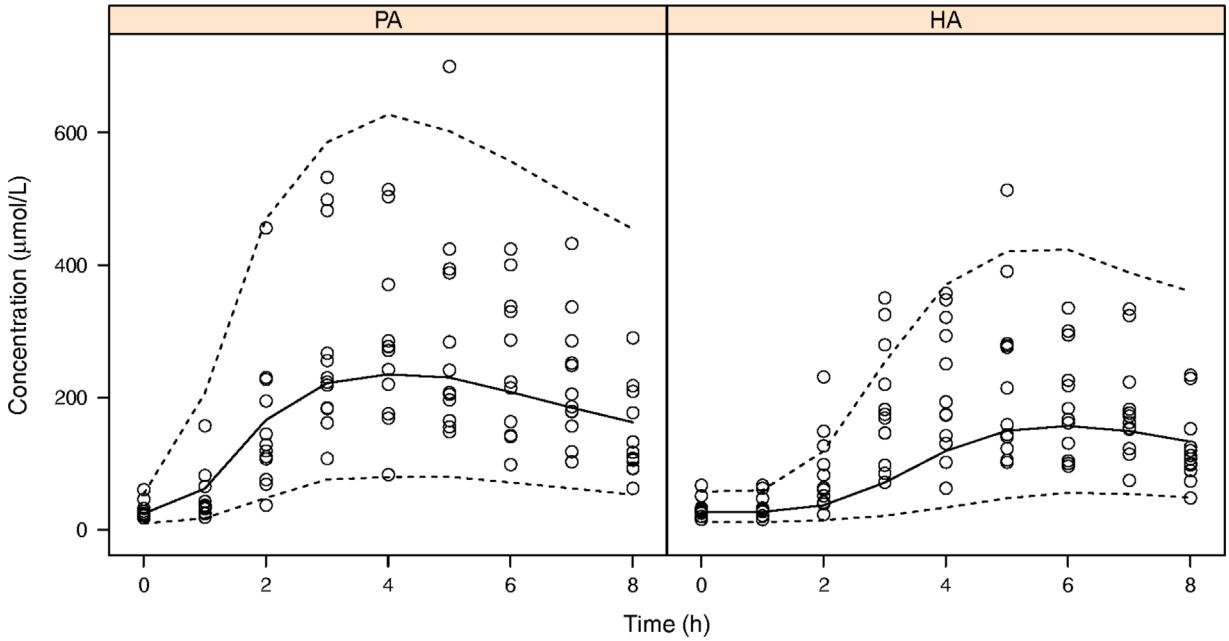

Figure 2. Visual predictive check for healthy subjects. Open circles are observed concentrations, solid line is the median from 500 simulated trials, dashed lines are the simulated 5th and 95th percentiles.

additional parameters, including the response to the timing of enzymes administration in subjects with CF. Model diagnostics demonstrated that the 1-compartment PK model with transit absorption model provided a reasonable description of PA and HA disposition. The visual predictive check further demonstrated that simulations from the final model yielded distributions that were representative of the observed data.

We have developed the MBT as a potential alternative to the CFA. Our initial experiments with the MBT were performed in healthy subjects who were made pancreatic insufficient using Orlistat, a lipase inhibitor.[17] The MBT detected fat malabsorption and differentiated between healthy subjects before and after Orlistat administration and subjects with CF and PI with and without enzyme administration. The extent of THA-to-HA absorption

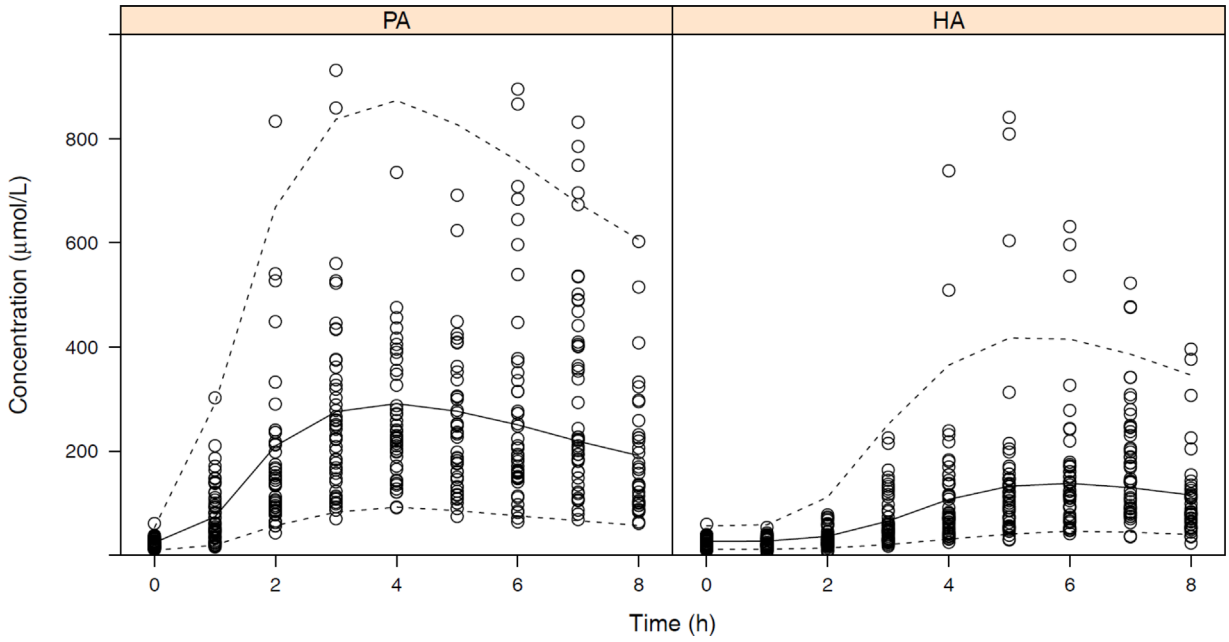

Figure 3. Visual predictive check for subjects with CF administered pancreatic enzymes with the MBT. Open circles are observed concentrations, solid line is the median from 500 simulated trials, dashed lines are the simulated 5th and 95th percentiles.

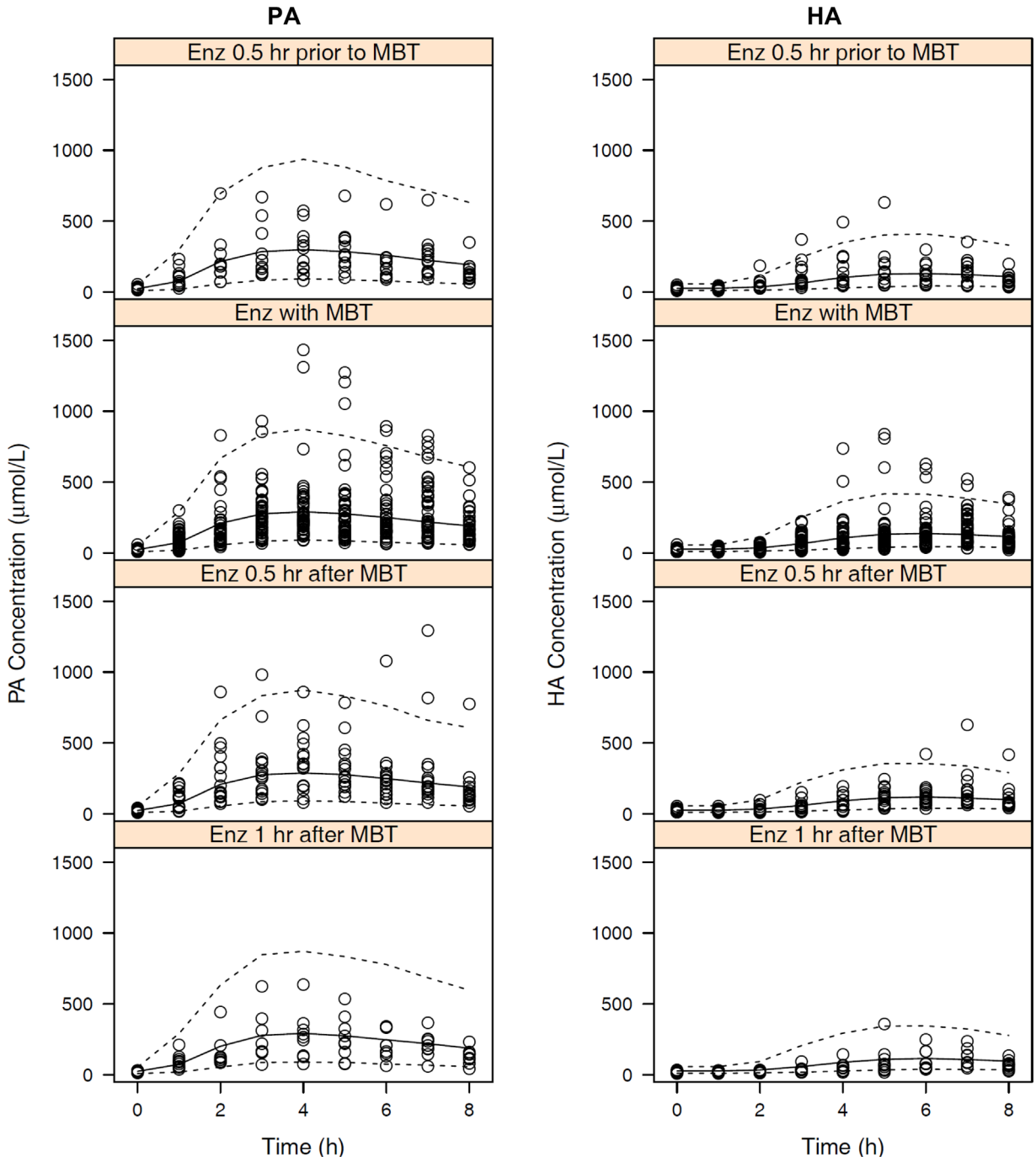

Figure 4. Visual predictive check for subjects with CF administered pancreatic enzymes at varying times relative to the MBT. Open circles are observed concentrations, solid line is the median from 500 simulated trials, dashed lines are the simulated 5th and 95th percentiles.

following administration of the MBT is indicative of its sensitivity to changes in fat malabsorption. After preliminary explorations with various PA and THA doses, we chose 5.0 g of PA and 5.5 g of THA for the MBT because these doses were more effective for detecting differences in fat absorption. PA and HA pharmacokinetics were variable in both the CF and healthy comparison groups. Although individual PA and HA profiles clearly demonstrated significant between-subject variability as well as between-occasion variability in exposure, this result was not surprising given the variable nature of the absorption process, compounded with alterations of the gastrointestinal tract in CF.[24]

With an expanded sample size for both the CF and healthy comparison groups, the results from this study confirms our previous findings that the MBT detects the

reduced THA digestion and absorption in subjects with CF and PI when pancreatic enzymes are not administered.[17] Here we have demonstrated that the MBT can detect more subtle differences in fat absorption based on the timing of enzyme ingestion around the meal. For years clinicians have instructed patients to administer enzymes before meals or at meal initiation with no data to support the practice, and many patients and families do not adhere to these directions for medication use.[25] It was in 1977 that DiMagno et al[26] first showed prandial administration of enzymes was as effective in decreasing fat loss as hourly enzyme administration. In adult patients with chronic pancreatitis, Dominguez-Munoz et al[27] in 2005 showed, using the ^{13}C mixed triglyceride breath test, that enzymes taken with meals or at the end of the meal improved fat absorption compared with when taken before meals. In contrast, Dorsey et al in 2010, showed in CF and PI that there was no difference in the CFA when enzymes were taken during meals or before meals.[9] The difference in these results may be related to the various methodologies used, diagnosis, disease severity, and that the CFA is not a highly reliable test. We have shown that for subjects with CF, PA was absorbed similarly to healthy subjects regardless of timing of enzymes. However, HA absorption was most similar to that of healthy subjects when enzymes were administered at the initiation of the meal or 30 minutes before. Absorption clearly diminished the longer enzyme administration was delayed relative to the initiation of the meal. These data provide evidence to support the current practice of prescribing enzyme use at the beginning of the meal. However, it should be noted that taking enzymes 30 minutes prior to the meal resulted in similar absorption, and this may be helpful in patients with CF who do not want to take their enzymes when in public with peers.

There are limitations to this study. Further studies are needed to expand the sample size to describe more definitively the enzyme timing effects of absorption using this modeling approach, ultimately leading to enhanced clinical applicability of the MBT. Further exploration of the MBT response in younger children and adults with CF will refine the modeling of the potential age effects or disease progression effects in CF not possible with our current study sample. The use of a standard pancreatic enzyme product for the MBT Protocols (Creon20, delayed-release, enteric-coated) may be seen as a limitation in interpreting the results of this study, as there are other similar products used by patients. The ratio of protease to lipase units in Creon 20 (3.8:1) is similar to that found in other products (ranges from 2:1 to 3.8:1). However, the generalizability of the results across all enzyme products is not known, but is likely generalizable as long as subjects are provided sufficient lipase units to optimize their fat absorption. The benefit of the use of 1 product in a research study was to lessen the between- and within-subject variability in the MBT. In addition, we did not standardize the enzyme dose for subjects with CF, as this may have caused increased malabsorption for some of the subjects requiring a higher enzyme dose. The present length of the MBT (9 hours) may limit its clinical application. Studies are planned to explore use of the MBT with fewer blood samples and a shorter sampling scheme. This should further improve use and acceptability of the test. The MBT may be helpful in assessment of pancreatic-based fat malabsorption in a broader range of diagnoses such as chronic pancreatitis and pancreatic cancer and in the frail elderly.

With the median age of survival of people with CF and PI increasing from 14 years in 1969 to 39 years in 2012,[1] CF is now both a pediatric and an adult disease. A significant component of the childhood growth failure (weight or height faltering) and poor nutritional status (reduced weight for height) in children and adults with CF is associated with PI.[28,29] Recently, Haupt et al[29] showed higher body mass index percentile in patients with CF treated at centers at which higher enzyme doses were used.[30]

Harris et al[31] established the important link between PI and malnutrition in children with CF more than 40 years ago by demonstrating that pancreatic enzymes improved dietary fat absorption and led to positive nitrogen balance. Steatorrhea and malabsorption are presenting symptoms in more than 20% of infants and children with CF. More than 87% of individuals with CF have PI and require lifelong pancreatic enzyme replacement therapy.[1] Therefore, an easier method for assessing degree of fat malabsorption in patients with PI has the potential for guiding enzyme therapy and refining nutritional intervention. The MBT promises to be a more reliable, specific, and quantitative clinical care test for the evaluation of malabsorption. The current one-size-fits-all therapy for administering enzymes may not be ideal for achieving maximum effectiveness of enzyme medication. The occurrence of fibrosing colonopathy[32] associated with high-dose enzyme exposure further underscores the importance of accurate documentation of the severity of malabsorption in CF care[33] and the use of enzymes at the minimum effective dose. Detecting more subtle differences in the degree of fat malabsorption may ultimately provide informative guidance for both pancreatic enzymes and nutritional intervention in children and adults with CF and PI. New enzyme products or modification of existing products require clinical and regulatory evaluation. A test that better discriminates the degree of malabsorption may improve this process and support new project development.

Conclusion

Based on these studies, and adding to the previous proof-of-concept findings, the MBT has promise as an

alternative to the CFA and the requirement of a 72-hour stool collection and diet record to describe dietary fat absorption. We have shown that the MBT detected changes in fat absorption when pancreatic enzymes were provided and when the timing of enzyme administration was altered. The MBT results showed that fat absorption was best when enzymes were administered with the onset of the meal or 30 minutes before the meal compared with 30 or 60 minutes after meal initiation. Further studies are needed to explore the sensitivity of the MBT to varying doses of enzymes and other malabsorption-related issues in the clinical care and research environment. The model-based approach described here provides a tool for evaluating MBT performance based on the degree of malabsorption documented. The key determinants explaining MBT variability provide a framework for future testing and guidance for application of the MBT.

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Declaration of Conflicting Interests

VAS is the inventor of the MBT. All other authors have no financial conflicts to disclose.

References

1. Cystic Fibrosis Foundation Patient Registry: 2012 Annual Data Report. Bethesda, MD: Cystic Fibrosis Foundation; 2013.
2. Wouthuyzen-Bakker M, Bodewes FA, Verkade HJ. Persistent fat malabsorption in cystic fibrosis; lessons from patients and mice. *J Cyst Fibros*. 2011;10:150–158.
3. Van de Kamer J, Huinick H, Weyers H. Rapid method for determination of fat in feces. *J Biol Chem*. 1949;177:347–355.
4. Weintraub A, Blau H, Mussaffi H, et al. Exocrine pancreatic function testing in patients with cystic fibrosis and pancreatic sufficiency: a correlation study. *J Pediatr Gastroenterol Nutr*. 2009;48:306–310.
5. Borowitz D, Konstan MW, O'Rourke A, Cohen M, Hendeles L, Murray FT. Coefficients of fat and nitrogen absorption in healthy subjects and individuals with cystic fibrosis. *J Pediatr Pharmacol Ther*. 2007;12:47–52.
6. Holmes GK, Hill PG. Do we still need to measure faecal fat? *Br Med J (Clin Res Ed)*. 1988;296:1552–1553.
7. Ishii Y, Kohno T, Ito A, et al. Measurement of extra-pancreatic secretory function by 13C-dipeptide breath test. *Transl Res*. 2007;149:298–303.
8. Caras S, Boyd D, Zipfel L, Sander-Struckmeier S. Evaluation of stool collections to measure efficacy of PERT in subjects with exocrine pancreatic insufficiency. *J Pediatr Gastroenterol Nutr*. 2011;53:634–640.
9. Dorsey J, Buckley D, Summer S, et al. Fat malabsorption in cystic fibrosis: comparison of quantitative fat assay and a novel assay using fecal lauric/behenic acid. *J Pediatr Gastroenterol Nutr*. 2010;50:441–446.
10. Schneider AR, Hammerstingl R, Heller M, et al. Does secretin-stimulated MRCP predict exocrine pancreatic insufficiency?: A comparison with noninvasive exocrine pancreatic function tests. *J Clin Gastroenterol*. 2006;40:851–855.
11. Ritz MA, Fraser RJ, Di Matteo AC, et al. Evaluation of the 13C-triolein breath test for fat malabsorption in adult patients with cystic fibrosis. *J Gastroenterol Hepatol*. 2004;19:448–453.
12. Laterza L, Scaldaferri F, Bruno G, et al. Pancreatic function assessment. *Eur Rev Med Pharmacol Sci*. 2013; 17(Suppl 2):65–71.
13. Vantrappen GR, Rutgeerts PJ, Ghoos YF, Hiele MI. Mixed triglyceride breath test: a noninvasive test of pancreatic lipase activity in the duodenum. *Gastroenterology*. 1989;96:1126–1134.
14. Keller J, Layer P, Bruckel S, Jahr C, Rosien U. 13C-mixed triglyceride breath test for evaluation of pancreatic exocrine function in diabetes mellitus. *Pancreas*. 2014;43:842–848.
15. van Dijk-van Aalst K, Van Den Driessche M, van Der Schoor S, et al. 13C mixed triglyceride breath test: a noninvasive method to assess lipase activity in children. *J Pediatr Gastroenterol Nutr*. 2001;32:579–585.
16. Herzog DC, Delvin EE, Albert C, Marcotte JE, Pelletier VA, Seidman EG. 13C-labeled mixed triglyceride breath test (13C MTG-BT) in healthy children and children with cystic fibrosis (CF) under pancreatic enzyme replacement therapy (PERT): a pilot study. *Clin Biochem*. 2008;41:1489–1492.
17. Stallings VA, Mondick JT, Schall JI, Barrett JS, Wilson M, Mascarenhas MR. Diagnosing malabsorption with systemic lipid profiling: pharmacokinetics of pentadecanoic acid and triheptadecanoic acid following oral administration in healthy subjects and subjects with cystic fibrosis. *Int J Clin Pharmacol Ther*. 2013;51:263–273.
18. Rovner AJ, Schall JI, Mondick JT, Zhuang H, Mascarenhas MR. Delayed small bowel transit in children with cystic fibrosis and pancreatic insufficiency. *J Pediatr Gastroenterol Nutr*. 2013;57: 81–84.
19. Beal SL, Sheiner LB. *NONMEM Users Guides*. San Francisco, CA: NONMEM Project Group, University of California at San Francisco; 1992.
20. Thai HT, Mentre F, Holford NH, Veyrat-Follet C, Comets E. Evaluation of bootstrap methods for estimating uncertainty of parameters in nonlinear mixed-effects models: a simulation study in population pharmacokinetics. *J Pharmacokinet Pharmacodyn*. 2014;41:15–33.
21. Yano Y, Beal SL, Sheiner LB. Evaluating pharmacokinetic/pharmacodynamic models using the posterior predictive check. *J Pharmacokinet Pharmacodyn*. 2001;28:171–192.
22. Savic RM, Jonker DM, Kerbusch T, Karlsson MO. Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. *J Pharmacokinet Pharmacodyn*. 2007;34:711–726.
23. Peeters MY, Allegaert K, Blusse van Oud-Alblas HJ, et al. Prediction of propofol clearance in children from an allometric model developed in rats, children and adults versus a 0.75 fixed-exponent allometric model. *Clin Pharmacokinet*. 2010;49: 269–275.

24. Borowitz D, Durie PR, Clarke LL, et al. Gastrointestinal outcomes and confounders in cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 2005;41:273–285.
25. Schall JI, Bentley T, Stallings VA. Meal patterns, dietary fat intake and pancreatic enzyme use in preadolescent children with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 2006;43:651–659.
26. DiMagno EP, Malagelada JR, Go VL, Moertel CG. Fate of orally ingested enzymes in pancreatic insufficiency. Comparison of two dosage schedules. *N Engl J Med*. 1977;296:1318–1322.
27. Dominguez-Munoz JE, Iglesias-Garcia J, Vilarino-Insua M, Iglesias-Rey M. 13C-mixed triglyceride breath test to assess oral enzyme substitution therapy in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol*. 2007;5:484–488.
28. Woestenenk JW, Stellato RK, Terheggen-Lagro SW, van der Ent CK, Houwen RH. The relationship between body growth and pulmonary function in children with cystic fibrosis. *Acta Paediatr*. 2014;103:162–167.
29. Peterson ML, Jacobs DR Jr, Milla CE. Longitudinal changes in growth parameters are correlated with changes in pulmonary function in children with cystic fibrosis. *Pediatrics*. 2003;112: 588–592.
30. Haupt ME, Kwasny MJ, Schechter MS, McColley SA. Pancreatic enzyme replacement therapy dosing and nutritional outcomes in children with cystic fibrosis. *J Pediatr*. 2014;164:1110–1115.
31. Harris R, Norman AP, Payne WW. The effect of pancreatin therapy on fat absorption and nitrogen retention in children with fibrocystic disease of the pancreas. *Arch Dis Child*. 1955;30: 424–427.
32. Smyth RL, Ashby D, O'Hea U, et al. Fibrosing colonopathy in cystic fibrosis: results of a case-control study. *Lancet*. 1995;346:1247–1251.
33. Borowitz D, Baker RD, Stallings V. Consensus report on nutrition for pediatric patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 2002;35:246–259.