Coefficient of Fat Absorption to Measure the Efficacy of Pancreatic Enzyme Replacement Therapy in People With Cystic Fibrosis

Gold Standard or Coal Standard?

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Objectives: We sought data on the validity, reliability, responsiveness, and feasibility of the coefficient of fat absorption (CFA) as a measure of pancreatic enzyme replacement therapy (PERT) efficacy in people with cystic fibrosis (pwCF) and reviewed the literature for alternative measures.

Methods: We searched PubMed for the Medical Subject Heading cystic fibrosis and the key words cystic fibrosis, fat absorption, CFA, and fecal fat imbalance; historical articles; and citations in bibliographies.

Results: The lower the CFA, the greater its variability; thus, it is less variable in healthy individuals who have higher CFA than pwCF. In addition, the test-retest values for CFA are more variable in pwCF than the general population. There is no correlation between CFA and body mass index or PERT dose but CFA is related to gastrointestinal signs and symptoms. Research-quality CFA studies are expensive, time consuming, and odious to pwCF and research staff. Sparse stool tests, breath tests, and blood tests of fat absorption have been studied as potential alternatives to CFA to measure PERT efficacy.

Conclusions: Based on the evidence, we conclude that CFA as a measure of the efficacy of PERT is more of a "coal standard" than a gold standard; developing suitable alternatives should be a priority.

Key Words: cystic fibrosis, enzyme replacement therapy, pancrelipase, exocrine pancreatic insufficiency, malabsorption, reproducibility of results

Early in the twentieth century, autopsy reports of patients with purulent lung infections and dramatic scarring and destruction of the pancreas led to the identification of a new disease entity, cystic fibrosis (CF).[1] Before the recognition that excessive secretion of chloride in sweat could be used as a diagnostic test for CF,

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direct measurement of duodenal enzymes was used to diagnose exocrine pancreatic insufficiency (EPI) as a way to identify children with this new disease.[2] There was debate among leading clinicians about how to treat people with CF (pwCF), most of whom died in infancy or early childhood, often as a direct consequence of malnutrition due to fat malabsorption. Opinions differed as to whether exogenous pancreatic extracts should be used as a therapy for CF, although ultimately this became an important contributor to extending survival.

Eventually, the coefficient of fat absorption (CFA) came to be used as the gold standard for diagnosis of EPI and as a measurement of treatment protocols to control fat malabsorption.[3–8] To calculate a CFA, the amount of fat in the diet is recorded and stool is analyzed for fat content. For a research-quality CFA, a diet is consumed that contains 100 g of fat each day over a 3-day period. Oral dye markers are ingested at the start and end of this period. All stools are collected from the appearance of first dye marker to second. Fat is measured in the pooled stool; the CFA (%) = 100 × ([grams fat intake − grams fat excretion]/grams fat intake). The accepted normal values are 85% or greater in infants up to 6 months of age and 93% or greater in children older than 6 months and adults.[9,10] Fat malabsorption also has been expressed as a coefficient of fat excretion, which is the inverse of CFA.

A gold standard, also known as a criterion standard, is the best diagnostic measurement for a condition against which new tests or results and protocols are compared. It should be valid, reliable, responsive, and ideally feasible (Fig. 1).

In practice, the criterion standard is rarely a perfect test but merely the best available test. Several recent reviews have examined the utility of CFA and other tests to diagnose EPI,[11–13] so that application of CFA will not be explored here. Our objectives are to review the literature for the second purpose, CFA as a measure of pancreatic enzyme replacement therapy (PERT) efficacy in pwCF, to assess its attributes for this purpose, and to determine whether other tests may improve upon these qualities.

MATERIALS AND METHODS

We developed and ran a PubMed search using the Medical Subject Heading cystic fibrosis and the key words cystic fibrosis, fat absorption, CFA, and fecal fat imbalance. We searched for the key words in the title and abstract fields. In addition, some historical articles were gleaned from one of the author's files (D.B.). The citations listed in some of the articles reviewed led to additional references.

RESULTS

Historical Context

By the 1950s, most key opinion leaders agreed that PERT should be used to treat pwCF. Clinicians sought ways to measure
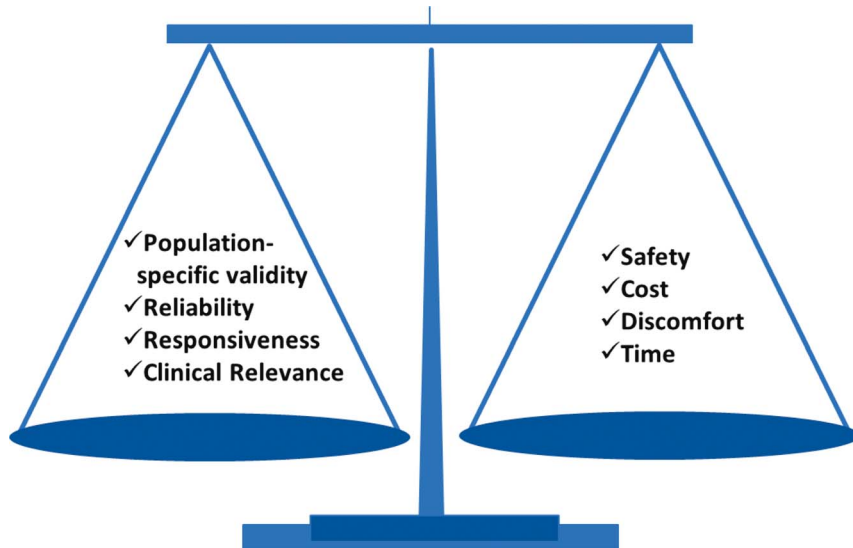
FIGURE 1. Factors to balance when choosing a criterion standard.

the efficacy of PERT.[14] In one early study of the effect of PERT by measuring fecal fat excretion, Harris et al[15] stated, "For many years substitution therapy with various preparations of the pancreas have been given to children with fibrocystic disease of the pancreas as a routine treatment… although the practice has been based on the clinical evaluation of the results rather than on any objective study of the results of metabolic balances." In 1958, van de Kamer[16] published a method to analyze wet stool to determine the fat content and thus provided the first standardized method to measure fat malabsorption. Of note, a nuclear magnetic resonance–based method is now widely in use; it produces the same results as the gravimetric "wet" method.[17] Once fat output in the stool is measured, a coefficient can be calculated by combining it with a careful measurement of fat intake. With his colleagues, van de Kamer[8] later published an article defining the use of CFA in clinical practice and described the conditions needed and the potential sources of error. They noted that "In order to decide if a steatorrhea exists or not, one has to pool the feces of at least 5 consecutive days… it is of advantage to calculate and note the sliding mean of 3 days in order to exclude, for the most part, the influence of daily fluctuations caused by the variability of intestinal motility" and diet. The authors demonstrated that there was greater variability in CFA, the lower it was (Fig. 2) and noted that the smaller the expected change, the greater the number of observations needed to assure that the change is significant, thus limiting the usefulness of CFA to detect change when PERT doses are changed.[8]

Coefficient of fat absorption may be most useful when large changes are expected, such as comparing the use of PERT with placebo. In 2004, in the wake of an epidemic of fibrosing colonopathy, an adverse effect likely due to excessive doses of PERT, the US Food and Drug Administration (FDA) required all PERT products be reformulated, submitted as a New Drug Application, and undergo regulatory review.[18] The agency provided guidance that PERT products be compared with placebo and recommended CFA as the test for malabsorption; 6 products were approved based on this measure (Table 1). Nonetheless, members of the Gastrointestinal (GI) Drugs Advisory Committee, upon reviewing one additional PERT, expressed concern about the limitations of CFA and its use as a surrogate marker or clinical measure.[19]

Attributes of CFA

Validity

A criterion standard should be valid, meaning that it is consistent with the hypothesis concerning the attribute that is measured and can be relied upon to measure accurately the attribute of interest. The CFA is deficient in both areas as a measure of pancreatic digestion. As a whole-gut, black box method, CFA measures fat malabsorption from all causes. It cannot distinguish fat malabsorption due to inadequate exogenous PERT or due to biliary, luminal, or epithelial causes.[20] For example, fat malabsorption is seen with biliary atresia, primary biliary cholangitis, sclerosing cholangitis, small intestinal bacterial overgrowth, and in short bowel syndrome. Celiac disease and radiation enteropathy cause fat malabsorption partly because of epithelial damage limiting absorption and partly because of lack of enteroendocrine signaling to the pancreas (secondary EPI). The CFA cannot distinguish whether the action of pancreatic enzymes is occurring proximally in the small intestine—the optimal site of action—or distally, because as a sample collected at the anus, it is an integrated measure of absorption or lack thereof in all parts of the intestinal lumen, including the contribution of colonic flora. In addition, it does not always accurately measure the attribute of interest. Indirect evidence from an older study that measured simultaneous CFA and coefficient of nitrogen absorption, values that track together, showed variability in coefficient of nitrogen absorption despite the fact that direct duodenal measurement of trypsin, chymotrypsin, and carboxypeptidase values were zero in all subjects.[21] Duodenal lipase values were not reported. This suggests that the variability of malabsorbed nitrogen in stool is due to factors other than pancreatic dysfunction and by implication, the same must be true for malabsorbed fat in the stool. Individuals with CF continue to secrete acid stable lingual lipase, which accounts for most of the endogenous lipase activity in pwCF.[20,21] An analysis of studies of PERT efficacy published in 2014 notes that in the absence of PERT (ie, the placebo arm of randomized studies), CFA measures endogenous lipase alone, whereas CFA on PERT measures both endogenous and the exogenous lipase activity of the PERT, introducing a small amount of bias when used as an end point to quantify PERT efficacy, which may or may not be clinically relevant.[22] Furthermore, in 2 studies that reported results when pwCF were
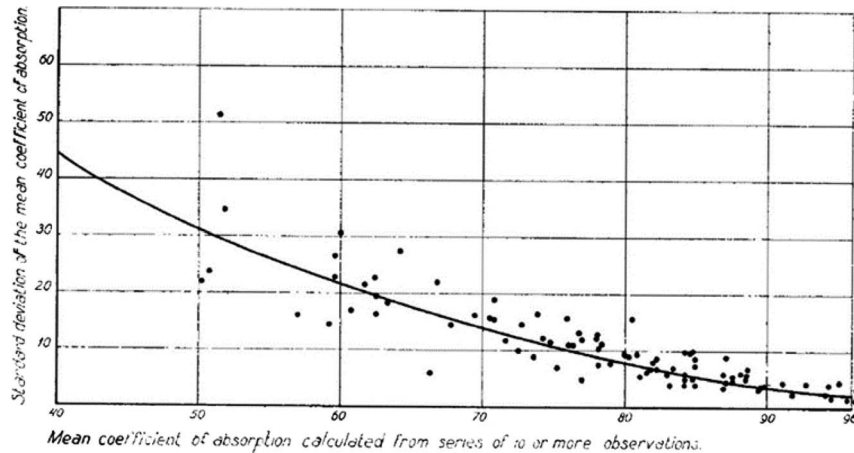
FIGURE 2. Variability of CFA is greater the greater the fat malabsorption (Reproduced with permission from Weijers et al,[8] 1960).

not taking PERT, the range of CFAs includes negative numbers, a physiologic impossibility.[23,24] These studies were performed by experienced investigators using research-quality methods. The inability of CFA to distinguish pancreatic from nonpancreatic causes of fat malabsorption, the confounding effect of endogenous lipase, and the reporting of nonphysiologic values for carefully performed CFA measurements suggest that CFA does not accurately measure exogenous pancreatic enzyme activity in the gut.

Reliability and Responsiveness

A criterion standard also should be reliable, that is, if repeated in the same person under the same conditions, the results should be the same. Between-test reproducibility is especially important when the purpose of the measurement is to identify changes due to a therapeutic intervention.[25] Reliability is a prerequisite for responsiveness, that is, the ability to detect change

because of an intervention known to affect the attribute of interest rather than as a consequence of variability in its test-retest properties. The between-test reproducibility of CFA is problematic. In one study when pwCF were taking PERT and had CFA repeated at 2 points in time, 41% of subjects (9/22) had a test-retest change equal or greater than 10 percentage points.[26] Two of these nine subjects had CFA in the normal range on PERT at one point in time but had a repeat value while taking the same dose of PERT that was clearly below normal. Five additional subjects with CFA less than 93% but greater than 80% had a value less than 80% at a second point in time.

Population issues are critical. In healthy subjects, the test-retest values for CFA have little variability, but CFA in CF subjects repeated at 2 points in time is highly variable, likely partially due to the large standard deviation in CFA when fat malabsorption is high (Fig. 2).[27] Even when examining large changes, such as the

TABLE 1. Pancreatic Enzyme Replacement Therapies With New Drug Applications Approved by the United States FDA Based on CFA

Commercial PERT Brand (Year Approved)	Current Distributor and Distribution and Manufacturing History	Comments
Creon (2009)	AbbVie, Inc. (North Chicago, Ill)	Enteric-coated porcine-derived pancrelipase
Zenpep (2009)	Nestlé HealthScience (Vevey, Switzerland) (ZenPep was originally developed by Eurand N.V.; Amsterdam, Netherlands)	Enteric-coated porcine-derived pancrelipase
Pancreaze (2010)	Vivus, LLC (Campbell, Calif) (Pancreaze was originally developed by Ortho-McNeil Pharmaceutical, LLC, now part of Janssen Pharmaceuticals, Inc, Raritan, NJ)	Enteric-coated porcine-derived pancrelipase
Ultresa (2012)	Aptalis Pharma US, Inc (Bridgewater, NJ, now a contract development and manufacturing organization rebranded as Adare Pharma Solutions, Lawrenceville, NJ)	Enteric-coated porcine-derived pancrelipase. The Ultresa brand name was discontinued in the US in 2016; FDA approval was withdrawn in 2019
Pertzye (2012)	Chiesi USA, Inc. (Cary, NC) (Pertzye was originally developed by and is still manufactured by Digestive Care, Inc., Bethlehem, Pa)	Enteric-coated porcine-derived pancrelipase with added bicarbonate.
Viokace (2012)	Nestlé HealthScience (Vevey, Switzerland) (Viokace had been distributed by Allergan plc, Madison, NJ, until 2020; Viokace was originally developed by Aptalis Pharma US, Inc., Bridgewater, NJ; now a contract development and manufacturing organization rebranded as Adare Pharma Solutions, Lawrenceville, NJ)	Non–enteric-coated porcine-derived pancrelipase tablets

An additional PERT was cleared by the FDA in 2015 as a device based on blood tests of fat absorption (Alcresta Therapeutics, Newton, Mass). This lipase-only cartridge is intended for external use in-line with enteric feedings.

comparison of CFA off PERT to CFA on treatment, the mean difference may be statistically significant, but the range of results can be exceptionally large. Although many authors do not publish those data, one recent well-conducted study reported that the range of treatment difference in CFA was between negative 7% and 75%.[3] Various studies have failed to demonstrate a clear difference among different PERT doses or use of acid blockade as an adjuvant to PERT when using CFA as an end point.[4,27–29] This lack of demonstrable dose response may represent true lack of difference with different doses or may be caused by the imprecision of the end point.[30] In other words, the test is not a sensitive measure of change or response. A Cochrane review of PERT for pwCF, updated in 2020, examined 14 trials involving more than 600 children and adults with CF, representing both parallel and cross-over trial designs. Interventions included different enteric and nonenteric-coated preparations of varying formulations in comparison with each other.[31] They did not find evidence on "the relative dosages of enzymes needed for people with different levels of severity of pancreatic insufficiency." Whether the wide range of CFA reported during the placebo arms of these studies actually reflects (1) different levels of pancreatic insufficiency, (2) the variety of other gastrointestinal confounders that can affect CFA in pwCF, or (3) measurement error is unclear. They expressed concerns that cross-over studies may underestimate the degree of inconsistency between the results due to overinflation of confidence intervals from the individual studies. In fact, the assertion that different dosages of enzymes are needed for people with different levels of severity of pancreatic insufficiency likely stems from the finding that CFA is highly variable. Because more than 90% of pancreatic enzyme production must be lost to have EPI, and autopsy and radiologic studies show almost complete destruction and fatty replacement of pancreatic parenchyma in pwCF, the range seen in CFA off PERT must represent something other than the EPI or PERT efficacy. The variability seen in the CF-EPI population and lack of test-retest stability in individuals precludes use of CFA for dose-response studies.

Correlation With Clinically Relevant Outcomes

Pancreatic enzyme replacement therapy clinical researchers have noted that "there is no consensus on what would constitute a clinically meaningful change in CFA."[5] Because CFA is a difficult test to perform, in practice, PERT dose is often prescribed or adjusted based on signs and symptoms. If patients with EPI are not treated with PERT, they have poor growth and may have flatulence, abdominal pain, and frequent fatty, loose stools. Despite this, among patients managed at a leading CF Center with GI and nutrition-focused care, investigators found no correlation between PERT dose and fat malabsorption (expressed as coefficient of fat excretion, the inverse of CFA; Fig. 3).[32]

Body mass index (BMI) is considered the main nutritional outcome measure in pwCF because it correlates with lung function and survival.[33] Based on the caloric density of fat, fat absorption should be strongly associated with the clinically relevant outcome of interest, which is weight and/or BMI: CFA does not seem to be valid in this aspect. An evidence-based review found "insufficient evidence to make a recommendation regarding the association of specific PERT dosing and CFA or growth."[33] Although one study using a large registry data set showed a statistical correlation between a small difference in PERT dose and BMI,[34] this difference is not achievable in practice for individuals because of the limitation in step-change dose per capsule of PERT. Many studies do not demonstrate a correlation between provider-prescribed PERT and BMI, including a prospective study where weight was the predefined end point.[4,21,28,35] Some authors have found an association between PERT use and improvement in GI signs and symptoms or stool frequency compared with placebo.[3,5,23,24] A retrospective review did not find a correlation between clinician-prescribed PERT dose and patient-reported symptoms nor was a correlation with parent-reported symptoms in infants and PERT dose demonstrated in a prospective study.[35,36] Although recent progress has been made, lack of a widely accepted standardized tool for GI-related outcomes and/or reporting of symptoms as adverse events in the past limits the utility of correlating symptoms
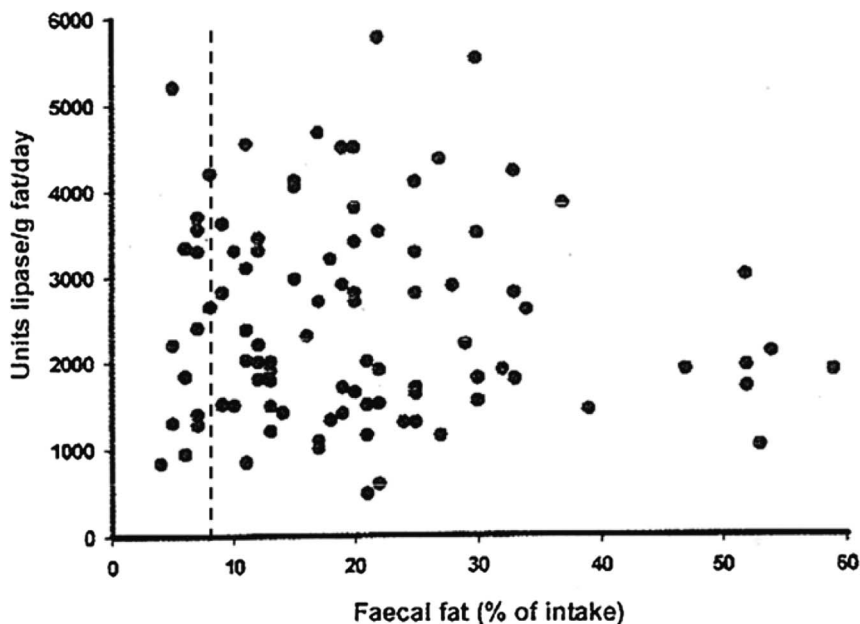

FIGURE 3. Lack of correlation between PERT dose and fat malabsorption expressed as coefficient of fat excretion (the inverse of CFA, reprinted with permission from Durie et al,[32] 1998). The dashed line represents a normal coefficient of excretion (<7%, the inverse of CFA); $r = -0.06$, $P > 0.5$.

with CFA.[37] In a study of patients with EPI secondary to chronic pancreatitis who do not have many of the other GI issues that confound studies in pwCF, those with a significant improvement in CFA on PERT had significant improvements in stool consistency and frequency, but not flatulence or abdominal pain.[38] Although improvements in stool signs and symptoms have been seen, neither CFA nor PERT dose is consistently correlated with growth, limiting the application of CFA as a surrogate marker of PERT efficacy for pwCF. Nonetheless, CFA has been deemed as an acceptable end point by the US FDA to support PERT efficacy.[39]

Practical Applicability

In addition to issues related to validity, reliability, responsiveness, and clinical relevance, a good criterion standard should be safe, acceptable to those who will be studied, and require minimal time and cost. A well-conducted fecal fat balance study requires an accurate recording of dietary intake and collection of all stools without any losses. To be of research quality, it also requires oral dye markers to delineate the stool that corresponds to the carefully measured 72-hour food intake. Although serious adverse events have not been reported, it has been posited that the requirement for 2 placebo (off-PERT) periods needed for parallel placebo-controlled trials has the potential to expose subjects to harm such as abdominal discomfort and caloric loss.[40] In a recent review, Fieker et al[41] state, "Currently there are no guidelines in clinical practice for monitoring the efficacy of enzyme replacement therapy and determining a need for dose adjustment… The cumbersome nature of stool studies limits their use…." Research-quality fecal fat balances studies, required for approval of PERT products, must be done in an inpatient setting. Even before COVID, these hospitalizations needed to follow strict infection protection and control guidelines to be safe for pwCF.[42] This need for repeated inpatient stays likely creates selection bias in those who were willing to commit to two 5- to 7-day hospitalizations. There are substantial drawbacks to these hospitalizations in time and cost, both financial and personal. Many consider the collection of stools to be repugnant. Notably, in an admirable study assessing a research-quality home method for fecal fat balance studies to explore PERT dosing, 10% of subjects dropped out because of the "severe stress" of stool collection.[43] Research-quality CFA studies are expensive, time consuming, and odious to pwCF and research staff.

Alternatives to CFA

Pancreatic enzyme replacement therapy is an essential part of treatment for pwCF, yet other than more precise manufacturing standards and the addition of bicarbonate to one PERT brand, there have been no novel advancements in PERT since the late 1970s. Methods to determine the efficacy of PERTs that may be developed and ways to adjust dose are needed. It has been said that "If you oppose, you must propose." Investigators and clinicians have long sought alternatives to fecal fat balance studies, using stool, breath, blood, and imaging studies. Although information about the validity, reliability, responsiveness, and clinical relevance is limited, we will review potential alternatives that minimize subject discomfort, time, and cost. A summary of the attributes of CFA and these alternatives is presented in Table 2.

Sparse Stool

Use of small aliquots of stool rather than a multiday collection ("sparse stool") has been proposed to avoid a complete fecal fat balance study:

- Single stool percent fat analysis: Caras et al[44] performed fecal fat balance studies and analyzed fat as a percentage of stool

dry weight using four different sparse stool methods assessed while research-quality CFA was being measured. Combined fat as a percentage of stool weight values on either the first or the last stool of each day that were below a 30% threshold were greatly predictive for CFA values equal or greater than 80% fat but were not useful below that level. Of note, this study used the ^{1}H-NMR method,[17] which provides fat measurements in terms of percentage of dry weight and has now supplanted the van de Kamer wet method for fecal fat analysis.

- Dysprosium partial pooled stool method: Another sparse stool method is to use dysprosium, an unabsorbed element, as a marker. There was a very high linear correlation of fractional excretion of dysprosium and ^{13}C-labeled triglyceride in individual stools in pwCF who exhibited a wide range of steatorrhea; thus, dysprosium alone could be used to measure fat excretion. Excretion based on the analysis of only the first 2 stools to appear after ingestion of an oral blue dye (partial pool method) was not different from those based on the analysis of all stools collected over 3 to 4 days.[45]

- Behenic acid stool test: A different method using an oral marker explored use of behenic acid, a nonabsorbable lipid marker. However, there was poor correlation with CFA.[46]

- Acid steatocrit: Steatocrit is the percent of fat in a capillary tube of centrifuged, homogenized stool; values for a healthy population have been published.[47] The addition of acid improves the method (acid steatocrit [AS]) and Tran et al[48,49] showed a high correlation between CFA and AS, but these results seem to be driven by those with well-controlled fat losses. Use of AS to define responders has been accepted by the FDA as an efficacy end point for children younger than 7 years.[6] In contrast, Wagner et al[50] did not find a strong correlation between AS and CFA. Walkowiak and colleagues[51] studied the use of AS compared with fecal fat excretion (the inverse of CFA) in pwCF being treated with PERT and concluded that its applicability in the assessment of fecal fat content in this subgroup of patients has limited practical value; this article is an excellent review of the AS literature.

In summary, sparse stool analysis may be useful depending on the method applied, especially in subjects where fat malabsorption is anticipated to be well controlled but has the same limitation as CFA in measuring whole-gut factors that lead to fat malabsorption, rather than just the contribution of pancreatic lipase.

Breath Testing

- ^{13}C-labeled triolein breath test: Collection of breath samples has been used to measure fat malabsorption, primarily in Europe. Triolein labeled with ^{13}C, a nonradioactive isotope, is mixed with a specified shake or meal. After digestion, absorption, and oxidation, the ^{13}C appears in breath carbon dioxide. Tidal breath collected at baseline and at regular intervals over 6 hours showed significant differences between healthy subjects and those with CF who are not taking PERT as well as between pwCF not taking PERT versus ingestion of the test meal with PERT.[52] These investigators noted that although delays in gastric emptying delayed the peak values, the area under the curve was not affected. No estimate was made of the between-test reproducibility in this small study of 6 adults with CF, although, as with CFA, the test was reproducible in healthy subjects. Herzog et al[53] also found repeatability in pwCF to be poor. Swart et al[54] studied adults with CF and identified sources of confounding, such as exercise and the ingestion of naturally ^{13}C-containing foods before testing. These investigators noted that "for a test

TABLE 2. Attributes of Short-Term Tests of Pancreatic Enzyme Activity

	Variability in a Healthy Population	Variability in the CF Population	Measures Only Pancreatic Enzyme Activity as a Cause of Fat Malabsorption	Correlates With a Clinically Relevant Outcome	Level of Financial and Opportunity Cost
CFA*	Low	High	No	+/−	High
Single stool percent fat analysis[†]	Low[†•]	High	No	+/−[†•]	Low[†•]
Dysprosium[‡]	ND	ND	No	ND	Low
Behenic acid[§]	Low	ND	No	ND	Low
Acid steatocrit[‖]	Low	High	No	ND	Low
[13]C breath test[¶]	Low	Moderate-high	No	ND	Low
MBT[#]	ND	ND	Yes	ND	Low
DECAT**	ND	ND	Yes	ND	Low

*Coefficient of fat absorption: 3-day 100-g fat diet demarcated by oral dye markers; stools collected from appearance of first dye marker to second; fat measured in pooled stool; CFA (%) = 100 × ([gram fat intake – gram fat excretion]/gram fat intake).

[†]Single stool percent fat analysis: A 5-g aliquot of stool collected during research-quality CFA is homogenized; stool fat is measured by nuclear magnetic resonance; the percent of fat was calculated based on the dry weight of each bowel movement.

• Imputed by the transitive property because the only data published was done during research-quality CFA determination and correlation with CFA was high.[44]

[‡]Dysprosium partial pooled stool method: dysprosium chloride and an oral blue dye marker are ingested with a high-fat meal; dysprosium is measured in the first blue stool and the subsequent stool.

[§]Behenic acid stool test: Sucrose polybehenate is mixed with fat-containing food and ingested; an aliquot of the next 2 to 3 stools is analyzed for lauric acid and behenic acid by gas chromatography; results are expressed as the ratio of the 2 acids.

[‖]Acid steatocrit: Stool is diluted with deionized water and homogenized; perchloric acid is added; this is aspirated into a capillary tube and centrifuged; AS = fatty layer length/(fatty layer length + solid layer length).

[¶][13]C-labeled triolein breath test: Triolein labeled with ^{13}C, a nonradioactive isotope, is mixed with a specified shake or meal; tidal breath is collected at baseline and at regular intervals over 6 hours; ^{13}C is measured by flow isotope ratio mass spectroscopy and is corrected relative to the Pee Dee Belemnite limestone international standard results, which are expressed as % dose recovered.

[#]Malabsorption Blood Test: A drink containing pentadecanoic acid and triheptadecanoic acid is ingested. Blood is collected before ingestion of the markers and then hourly over 8 hours.

**DHA + EPA Challenge Absorption Test (DECAT): A drink or meal containing DHA and EPA is ingested. Blood is collected before ingestion of the markers and then at intervals over 24 hours to calculate an area under the curve.

ND indicates no data.

that is intended to be used for clinical decision making, (the) degree of reproducibility is reasonable but less than desirable." Another study used ^{13}C breath tests to study response to different doses of PERT in infants and toddlers and performed simultaneous outpatient CFA. Neither method showed dose-response differences.[29] An earlier study reported that 20% of subjects studied did not have a change in ^{13}C measurements when their PERT dose was doubled.[55] [13]C breath testing is noninvasive and convenient but is not precise enough to become a criterion standard.

Blood Tests

Methods have been described that hold the potential to quantify more precisely the activity of PERTs, including the timing of action: in essence, they are pharmacokinetic tests. In contrast to whole-gut measures of fat malabsorption, these tests aim to evaluate the contribution of pancreatic lipase: a tracer that requires digestion by pancreatic lipase is provided orally and appearance of the tracer in blood is then measured.

• Malabsorption blood test: As described by the authors, "the malabsorption blood test (MBT) utilizes two naturally occurring fatty acids with odd-number carbon atoms chain length: pentadecanoic fatty acid (PA) and triheptadecanoic acid (THA). Pentadecanoic acid, a free fatty acid, is absorbed without need for pancreatic lipase, whereas THA, a triglyceride, requires hydrolysis by lipase to heptadecanoic acid before absorption."[56] Blood is collected before ingestion of the markers and then hourly over 8 hours. Mascarenhas et al[57] studied healthy subjects and those with CF and evaluated pharmacokinetic responses to timing of PERT ingestion relative to meals. Triheptadecanoic acid absorption, the measure of lipase activity, was most like that of healthy subjects when enzymes were administered at the initiation of the meal or 30 minutes before, rather than after the meal, showing that the method was able to detect differences in therapeutic interventions. The test-retest characteristics showed that PA and THA absorption was moderately variable within CF subjects. The MBT may be more sensitive than CFA to detect change. In a study of MBT in individuals with chronic pancreatitis and EPI, there was no significant change in mean CFA, but absorption of heptadecanoic acid increased significantly after PERT in subjects with chronic pancreatitis, while PA did not change.[7]

• DHA (docosahexaenoic acid) + EPA (eicosapentaenoic acid) Challenge Challenge Absorption Test: An alternative pharmacokinetic-like blood test, the DHA + EPA Challenge Absorption Test, measures the change in plasma concentrations of the long-chain, polyunsaturated fatty acids, DHA, and EPA as markers of fat absorption. Diet is the primary although limited source of absorbed DHA and EPA. Because less than 1% of these fatty acids are synthesized in the body, an oral marker can be given and its uptake can be studied. Most pwCF have levels of plasma DHA well below those seen in a healthy population.[58–60] An

omega-3-enriched oral marker can be given and blood collected over time to generate a peak and area under the curve relative to baseline. These long-chain polyunsaturated fats are more difficult to absorb than fats with a shorter carbon chain length; therefore, demonstration of plasma levels after ingestion of an enriched aliquot is a robust biomarker of global fat absorption. Measurement of plasma DHA and EPA by ultrahigh-performance liquid chromatography requires a minimal blood volume, an especially important factor in pediatric studies. The DHA + EPA Challenge Absorption Test was used to study the efficacy of a lipase-only in-line enzyme cartridge used with enteral tube feedings. Notable increases in levels were seen between 3 and 5 hours after use of the lipase and the area under the curve was significantly different than levels in pwCF-taking placebo.[61] In summary, pharmacokinetic-like blood tests to measure PERT efficacy have the advantages of measuring onset and duration of activity, being a more targeted measure of the contribution of pancreatic enzyme activity to fat absorption, not requiring inpatient stays, and being shorter in duration than CFA. Collection of multiple blood samples may not be acceptable to some pwCF, although the use of an indwelling catheter to draw samples may minimize this concern. Because these tests have the potential to be more responsive to measure the effects of therapeutic interventions than CFA, research evaluating the repeatability of these tests would be invaluable to the field.

Imaging

- Secretin-stimulated magnetic resonance cholangiopancreatography: This test is being pursued as an exocrine pancreatic function test, with the degree of ductal dilation in response to secretin considered a surrogate marker for ductal function, although it does not measure acinar function.
- Magnetic resonance imaging (MRI): Magnetic resonance imaging can distinguish fat from water, using precontrast T1, nonfat-suppressed sequences and T2 fat-suppressed images, respectively. Thus, it has the potential to quantify malabsorbed fat in the small intestine. Increased small bowel water content in pwCF has been demonstrated by MRI.[62] It remains to be seen whether MRI can become an alternative way to measure fat absorption and thus PERT efficacy.

Measurement of Long-Term Efficacy of PERT

The CFA and the potential alternatives outlined previously can measure the efficacy of PERT only over a short period. The need for PERT lasts a lifetime in people with EPI; therefore, studies of long-term effect are needed. As noted previously, the most relevant clinical outcome to prove that pancreatic enzyme activity function has been adequately supported by PERT is growth. Unfortunately, weight gain or lack thereof can be caused by many factors other than the contribution of pancreatic digestion. Similarly, GI symptoms may have a range of etiologies. Ideally, an objective test that is specific to digestion and absorption of fat would be available to complement short-term studies of PERT efficacy and correlate with signs and symptoms relevant to long-term PERT use, analogous to blood glucose measurements and hemoglobin A1C.

- Red blood cell (RBC) membrane lipid analysis: One way to measure chronic fat absorption is to measure fatty acids incorporated into RBC membranes. In addition to EPA and DHA, 22 additional long-chain polyunsaturated, saturated, monounsaturated, and trans-unsaturated fats can be measured. The sum of these 24 fatty acids constitutes the total fatty acid content of the RBC membrane, and each individual fatty acid can be expressed as a percent of the total or as a concentration in micrograms per milliliter. In healthy adult women, total trans fatty acids and total 18:1 trans isomers in RBC membranes were strongly correlated with carefully recorded intake; of all fatty acids measured, DHA provided the strongest correlations with its consumption.[63,64] An objective test of chronic fat absorption, such as RBC membrane lipid analysis, that could be correlated with growth and GI symptoms would be a significant advance in proving the clinical relevance of PERT dosing.

- Omega-3 index (O3I): EPA and DHA typically comprise approximately 3% to 5% of erythrocyte fatty acids in Western populations with low fish intakes. Red blood cell membrane lipid analysis has been shown to be a marker of omega-3 fatty acid absorption over a 12-week period in patients with maldigestion and malabsorption due to a variety of gastrointestinal causes.[64] Measurement of DHA and EPA in finger stick blood spots, termed the O3I, has been used to assess intake of omega-3 fatty acids and is commercially available.[65] The coefficient of variability of the O3I in the healthy population is within the range of other commonly used laboratory tests such as calcium, glucose, cholesterol, and uric acid and is unaffected by recent meal intake.[66,67] Many studies have focused on omega-3 fatty acids in cell membranes and the risk for cardiovascular disease, thus establishing the range of values in the general adult population.[68–70] Measurement of RBC omega-3 fatty acids has been reported in pwCF who used a lipase-only enzyme cartridge to support absorption of enteral tube feedings and showed steady increases in absorbed DHA and EPA over a 90-day period.[71] As noted previously, the baseline levels for DHA and EPA in pwCF, including children, are significantly lower than those seen in the healthy population.[58–60] In most individuals, dietary intake is relatively stable. If a new PERT were introduced or dose changed and the O3I increased over several months, it could demonstrate that the PERT led to improved fat absorption. The potential for an at-home finger stick test that could be shipped to a central laboratory for analysis holds promise for studies of long-term fat absorption in pwCF.

DISCUSSION

In a Letter to the Editor, Versi[72] states, "Gold standards are constantly challenged and superseded when appropriate." Based on the evidence presented previously, we conclude that CFA as a measure of the efficacy of PERT is more of a "coal standard" than a gold standard. Just as coal generated the energy that fueled the Industrial Revolution, CFA has powered our early understanding of pancreatic insufficiency and PERT efficacy. However, the thermodynamic efficiency of coal-powered energy plants varies between approximately 32% and 42%.[73] Similarly, CFA is an inefficient way to understand PERT efficacy. In our present world, CFA, the current criterion standard, should be supplanted by other more modern outcomes, especially those that mimic pharmacokinetic tests, to answer questions about dose response and the timing and duration of action of PERT in addition to measuring PERT efficacy. The development of an objective test to measure long-term PERT effectiveness as a complement to short-term tests of efficacy would be a significant step forward in improving our use of PERT. Alternatives to CFA as a measure of PERT efficacy must demonstrate population-specific validity, reliability, responsiveness, and clinical relevance and should be safe and require minimal cost, discomfort, and time. Research to establish these factors for some of the alternative measures noted here should be a priority to fuel a better future for pwCF.

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