

Diagnosing Pancreatic-Based Malabsorption in Patients with Chronic Pancreatitis

AbbVie Inc.

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Lead Investigator/ Study Physician

Virginia A. Stallings, MD
3535 Market Street Rm 1558
Philadelphia, PA 19104
Phone 215-590-1664
email: stallingsv@email.chop.edu

Principal Investigator

Babette Zemel, PhD
3535 Market Street Rm 1558
Philadelphia, PA 19104
Phone 215-590-1669
email: zemel@email.chop.edu

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ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
BC	Bomb calorimetry
BMI	Body mass index
CF	Cystic fibrosis
CFA	Coefficient of fat absorption
CP	Chronic pancreatitis
Creon36™	Creon (pancrelipase) Delayed-Release Capsules: 36,000 USP units of lipase
CTRC	Clinical Translational Research Center
DXA	Dual energy x-ray absorptiometry
FFM	Fat free mass
FM	Fat mass
HA	Heptadecanoic acid
MBT	Malabsorption blood test
NDS	Nutrition Data System
PA	Pentadecanoic acid
PHI	Personal health information
PIVKA	Proteins induced by vitamin K antagonism or absence
PROMIS	Patient reported outcomes
QOL	Quality of life
SAE	Serious adverse event
THA	Triheptadecanoic acid

ABSTRACT

Context: Reduced exocrine pancreatic function or pancreatic insufficiency (RPF/PI) contributes to poor clinical outcome in a number of diseases and conditions. The incidence, mechanism and substantial adverse clinical outcomes of PI are well known in patients with cystic fibrosis (CF), and the life sustaining role of pancreatic enzyme medication in CF care is well established¹⁻³. Much less is known about the incidence and impact of RPF/PI in patients with chronic pancreatitis (CP). Reliable non-invasive screening or diagnostic tests with acceptable patient burden are not available for RPF/PI.

Objectives: The objective of this study is to evaluate the malabsorption blood test (MBT), stool coefficient of fat absorption (CFA) and stool bomb calorimetry (BC) methods as potential screening or diagnostic tests for RPF/PI. A further objective is to determine the test responses before and after pancreatic enzyme medication administration (Creon36™) in the patients with CP. We will also explore the association of specific genotypes with fat malabsorption in the context of CP.

Study Design: This is a cohort study of subjects with CP who will be evaluated before and after pancreatic enzyme medication (Creon36™) administration. A cohort of healthy subjects will serve as a comparison group and will be evaluated only once.

Setting/Participants: This outpatient study will be conducted at CHOP. Twenty four subjects with CP and evidence of being at-risk for malabsorption, age 30-75 years, and 24 healthy comparisons, age 30-70 years, with no known chronic disease that would affect dietary or fat absorption will be enrolled.

Study Interventions and Measures: Subjects with CP will receive Creon36™, a pancreatic enzyme medication, and fat and energy absorption will be evaluated using three methods: MBT, CFA, and BC before and after administration of Creon36™. Many patients with CP are at risk for RPF/PI yet they rarely undergo diagnostic testing. Pancreatic enzyme medication will likely improve clinical outcomes and quality of life in some of those with RPF/PI. A cohort of healthy volunteers will be evaluated with the three methods to provide essential comparison data to optimize the understanding and interpretation of the findings from the three methods and the RPF/PI cohort with CP. There will be no intervention for the healthy cohort.

PROTOCOL SYNOPSIS

Study Title	Diagnosing Pancreatic-Based Malabsorption in Patients with Chronic Pancreatitis
Funder	AbbVie Inc.
Clinical Phase	Phase II – This is an open-label intervention therapeutic exploratory study
Study Rationale	<p>Reduced exocrine pancreatic function or pancreatic insufficiency (RPF/PI) contributes to poor clinical outcome in a number of diseases and conditions. The incidence, mechanism and substantial adverse clinical outcomes of PI are well known in patients with cystic fibrosis (CF), and the life sustaining role of pancreatic enzyme medication in CF care is well established¹⁻³. Much less is known about the incidence and impact of RPF/PI in patients with chronic pancreatitis (CP). Reliable non-invasive screening or diagnostic tests with acceptable patient burden are not available for RPF/PI. We propose a study to evaluate the malabsorption blood test (MBT), stool coefficient of fat absorption (CFA) and stool bomb calorimetry (BC) methods as potential screening or diagnostic tests for RPF/PI. In subjects with CP, we also propose to administer pancreatic enzyme medication (Creon36™) to determine the test responses before and after pancreatic enzyme medication administration in these patients. Many patients with CP are at risk for RPF/PI yet they rarely undergo diagnostic testing. Pancreatic enzyme medication will likely improve clinical outcomes and quality of life in some of those with RPF/PI. A cohort of healthy volunteers will be evaluated with the three methods to provide essential comparison data to optimize the understanding and interpretation of the findings from the three methods and the RPF/PI cohort with CP.</p>
Study Objective(s)	<p>Primary</p> <p>Utilizing the MBT, CFA and BC methods to identify RPF/PI in subjects with CP, our primary hypotheses are:</p> <p>H1: Subjects diagnosed with CP and not receiving pancreatic enzyme medication will have RPF/PI as indicated by the reduced MBT, CFA and BC fat and energy absorption pattern compared to healthy participants.</p> <p>H2: Subjects with CP with reduced RPF/PI by the MBT, CFA and BC fat and energy absorption pattern will have improved fat absorption after pancreatic enzyme medication (Creon36™) as indicated by the MBT, CFA and BC increased fat and energy absorption pattern.</p> <p>Secondary</p> <p>The secondary objective of this proposal is to utilize these data to optimize the MBT design and application by reducing the time and blood samples required.</p> <p>H3: The MBT will be optimized to identify RPF/PI with 3-4 blood samples (vs 9 current samples) over 4-5-hours (vs current 8-hours).</p>

	<p>We will explore the utility of the MBT compared to the CFA and BC (both require the burdensome 72-hour total stool and dietary intake collection) in identifying differences in fat absorption between subjects with CP and healthy comparisons and within the CP cohort, with and without pancreatic enzyme medication. We will also collect saliva for genetic testing, in order explore the association of specific genotypes with fat malabsorption in the context of CP.</p>
Test Article	Creon36™, a pancreatic enzyme preparation
Study Design	We propose to characterize pancreatic-based fat malabsorption in subjects with chronic pancreatitis (CP) compared to a healthy comparison group. Furthermore, we propose a longitudinal study design to assess pancreatic-based fat absorption before and after pancreatic enzyme medication in subjects with CP.

Inclusion and Exclusion:
INCLUSION CRITERIA**Chronic Pancreatitis Cohort**

- Chronic pancreatitis diagnosis by gastroenterologist. Participants with CP will be characterized based on the TIGAR-O (toxic, genetic, autoimmune, recurrent, obstructive) etiology system, on pancreatic morphology (Cambridge criteria) when available, and on physiological state (exocrine and endocrine function) as recommended by the recent American Pancreatic Association Practice Guidelines⁴.
- Age 30-75 years old
- Evidence of at-risk for malabsorption including: 1) history of use of and response to pancreatic enzyme medication; 2) history of unintentional weight loss; 3) history of increased stools per week or fatty stools; and/or 4) other clinical signs or symptoms suggestive of fat malabsorption
- In usual state of health for past two weeks including no change in medications
- Able to consume a moderate fat diet for stool evaluations
- Able to participate in the study for about four weeks with two study visits

Healthy Cohort

- Age 30-70 years old
- No known chronic disease that would affect dietary intake or fat absorption
- In usual state of health for past two weeks, with stable medications, diet and weight
- BMI from 14-35
- Able to consume a moderate fat diet for stool evaluations
- Able to participate in the study for about one week with one study visit

EXCLUSION CRITERIA**Chronic Pancreatitis Cohort**

- Evidence of normal fat absorption in medical record
 - Medications that alter fat absorption (i.e. orlistat, other weight loss medications, ursodeoxycholic acid)
 - Allergy to pork products
 - History of intestinal blockage or fibrosing colonopathy
 - Current diagnosis of gout and treatment with allopurinol medical therapy
- Pregnancy or breast feeding

Healthy Cohort

- Evidence of fat malabsorption
- Medications that alter fat absorption (i.e. orlistat, other weight loss medications, ursodeoxycholic acid)
- Pregnancy or breast feeding

Number Of Subjects	24 subjects with CP
	24 healthy subjects

Study Location	All study visits will occur at outpatient research offices at the Clinical and Translational Research Center of the Children’s Hospital of Philadelphia (CHOP), a pediatric health care center.
Study Duration	CP subject participation will last about four weeks Healthy subject participation will last about one week
Study Phases	<u>Screening</u> : screening for eligibility and obtaining consent <u>Visit 1</u> : First study visit at CHOP for CP (pancreatic enzyme medication -naive) and Healthy subjects <u>Visit 2</u> : One to four weeks after end of stool collection for first visit, a follow-up visit at CHOP for CP subjects only after three-day pancreatic enzyme medication treatment
Efficacy Evaluations	The efficacy of pancreatic enzyme medication administration (Creon36™) will be evaluated in subjects with CP using MBT, CFA% and BC outcomes
Pharmacokinetic Evaluations	<u>N/A</u>
Safety Evaluations	Safety will be monitored by adverse events reporting. The frequencies of AEs by type, body system, severity and relationship to study supplement will be summarized. SAEs (if any) will be described in detail.
Statistical And Analytic Plan	The goal of the primary aims is to 1) describe pancreatic-based fat malabsorption in subjects with CP using MBT, SFA% and BC outcomes compared to healthy subjects, and 2) compare these outcomes before and after pancreatic enzyme medication administration in subjects with chronic pancreatitis. Descriptive statistics for MBT, CFA% and BC outcomes (mean, standard deviation, median, range, 95% CI) will be calculated for the CP cohort and for the healthy comparison group, and differences between CP and healthy groups assessed with unpaired t tests. For the MBT outcomes, a moment-based pharmacokinetic (PK) analysis will be performed based on non-compartmental methodology using WinNonLin version 9.1 (Pharsight, Cary, NC). Baseline (C_0) and maximum (C_{max}) plasma concentrations are calculated, and area under the curve from time zero to eight hours (AUC) is calculated using the linear trapezoid method. PK parameters can then be compared between treatment groups using a paired t-test or Wilcoxon signed rank test as appropriate. Alternately, population PK analyses for repeated-measures endpoints can be conducted via nonlinear mixed-effects modeling. Descriptive statistics for MBT, CFA% and BC outcomes (mean, standard deviation, median, range, 95% CI) will also be calculated for the CP cohort with and without pancreatic enzyme medication administration, and the significance of changes over time assessed with paired t tests. A mixed effects modeling approach will be employed to examine the correlation of subject characteristics (age, BMI, sex, disease status, etc.) to PA and HA exposure metrics derived from the non-compartmental analysis to assess change over time in MBT response to pancreatic enzyme medication administration in subjects with CP.

Pharmacokinetic modelling will be employed to identify the three to four blood samples over the first four to five hours of the MBT that provide the most informative and most reproducible estimate of the full 8-hour MBT (nine blood samples) in the CP and healthy cohorts. The goal is a shorter sampling scheme for the MBT to optimize its use as a clinical diagnostic test. In exploratory analyses, we will compare the methods for detection of fat malabsorption as measured by CFA% and BC with fat absorption as measured by MBT for the subjects with CP compared to healthy individuals and also within the CP cohort before and after pancreatic enzyme medication use. A correlation analysis will be conducted to evaluate the performance of the MBT relative to that of the CFA%. HA and PA C_{max} , AUC, and HA/PA ratios will be plotted against CFA% or BC caloric loss to explore the relationship between the two tests. Any relationships observed in the plots will be further explored using correlation analysis and appropriate regression analysis as dictated by the observations. If a reasonable model can be developed to describe the response of MBT, CFA% and BC response to pancreatic enzyme medication treatment within the CP cohort, additional simulation studies will be performed to further examine the performance characteristics of both tests.

MONITORING PLAN

The Principal Investigator (PI) (Dr. Zemel) is ultimately responsible for monitoring data integrity and patient safety and for overall study oversight. The study will be monitored weekly by the PI and Lead Investigator (LI)/ Study Physician (Dr. Stallings). The study protocol will be carried out in accordance with OHRP guidelines and requirements. SAEs that are unanticipated, serious, and possibly related to the study will be managed by the PI and LI/ Study Physician in consultation with the participant's adult gastroenterology physician team (Dr. Chandrasekhara (Perelman Center for Advanced Medicine/HUP, [Penn Medicine/HUP]) or Dr. Rajala (Penn Medicine/ Philadelphia Veterans Affairs Medical Center [Penn Medicine/VA Hospital]) who will be immediately notified and will assume acute care management. Dr. Stallings will also consult with Dr. Mamula (CHOP/GI) who will be notified if an unexpected acute event occurs during a study visit at CHOP. In the case of AEs and SAEs arising in healthy subjects, the event will be managed by the PI and Dr. Stallings (the LI/ Study Physician) in consultation with the primary care physician identified by the participant upon enrollment in the study, who will be immediately notified. SAEs will also be reported to the study sponsor, IRB, CTRC, and all members of the research team in accordance with requirements. Anticipated SAEs or those unrelated to the study will be reported to the same individuals/entities in accordance with requirements. There will be ongoing collection of data on adverse events and compliance to the treatment protocol throughout the study by research staff and which will be reviewed weekly by the PI and the LI/ Study Physician (Dr. Stallings).

SCHEDULE OF STUDY PROCEDURES & STUDY TIMELINE

Table 1	Cohort with CP		Comparison Group Visit 1
	Visit 1 Without Creon36™	Visit 2 With Creon36™	
<i>Assessment</i>			
Study Intervention: Administration of Creon36™		√	
Primary outcome			
Malabsorption Blood Test (MBT) (PA, HA, HA/PA absorption, pharmacokinetics)	√	√	√
Secondary Outcomes			
72 hour Stool Fat: coefficient of fat absorption (CFA)	√	√	√
Fecal Bomb Calorimetry (BC)	√	√	√
Fecal Elastase 1	√		
Serum Fat Soluble Vitamins (A,D,E,K), Pre-albumin	√	√	√
Fatty Acid panel	√	√	
Serum Zinc and Selenium	√	√	√
Genetics			
DNA Sequencing (PRSS1, CTFC, CFTR, SPINK1) and others	√		
Diet			
3-Day Weighed Food Record	√	√	√
Safety			
Serum Comprehensive Metabolic Panel (CMP)	√	√	
Complete Blood Count (CBC) w/ Differential	√	√	
Adverse Event Monitoring	√	√	√
Other			
Anthropometry: Height, Weight, Skinfolds, Circumferences	√	√	√
Whole body DXA: body composition	√		
Home Environment Questionnaire	√		√
Health History and Medications	√		√
Quality of Life (MOS SF-36) and PROMIS survey	√		√

Table 2. Study Timeline

Month of study	Year 1 (2016)												Year 2 (2017)				Total																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16																									
Start-up	X	X	X																																						
Healthy Controls																																									
Baseline					2	3	3	3	3	3	3	2	2																												
CP group																																									
Baseline					2	3	3	2	2	2	2	3	3	2																											
Follow Up					2	3	3	2	2	2	2	3	3	2																											
Visit Total					6	9	9	7	7	7	7	8	8	4																											
Analysis												X	X	X	X	X	X																								
Closeout																	X	X																							

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Reduced exocrine pancreatic function or pancreatic insufficiency (RPF/PI) contributes to poor clinical outcome in a number of diseases and conditions. The incidence, mechanism and substantial

adverse clinical outcomes of PI are well known in patients with cystic fibrosis (CF), and the life sustaining role of pancreatic enzyme medication in CF care is well established¹⁻³. Much less is known about the incidence and impact of RPF/PI in patients with chronic pancreatitis (CP). Reliable non-invasive screening or diagnostic tests with acceptable patient burden are not available for RPF/PI. We propose a study to evaluate the malabsorption blood test (MBT), stool coefficient of fat absorption (CFA) and stool bomb calorimetry (BC) methods as potential screening or diagnostic tests for RPF/PI and to determine the test responses to pancreatic enzyme medication compared to responses with pancreatic enzyme medication-naïve status in patients with CP. Many patients with CP are at risk for RPF/PI yet they rarely undergo diagnostic testing. Pancreatic enzyme medication will likely improve clinical outcomes and quality of life in those with RPF/PI. A cohort of healthy volunteers will be evaluated with the three methods to provide essential comparison data to optimize the understanding and interpretation of the findings from the three methods and the RPF/PI cohort with CP. All subjects will be seen at the Children's Hospital of Philadelphia's outpatient Clinical Translational Research Center.

1.2 Name and Description of Investigational Product or Intervention

CREON (pancrelipase) Delayed-Release Capsules 36,000 USP units of lipase (Creon36™), a pancreatic enzyme preparation, is a drug that requires prescription for use. It is approved for use, including the current indication and dosage, by the FDA.

1.3 Findings from Non-Clinical and Clinical Studies

N/A – This is an FDA approved drug

1.4 Selection of Drugs and Dosages

Subjects with CP will take Creon36™ for nine days, and the daily dose selected is 72,000 lipase units per meal (two capsules) and 36,000 units per snack (one capsule), with each Creon capsule containing 36,000 lipase units. Subjects will take Creon36™ for three days prior to Visit 2, the day of Visit 2 and then for five days after the visit until they have completed stool collections. The pancreatic enzyme medication dose range for adults with CP is not well standardized. The dose chosen for these short term studies are in the higher range recommended for initial treatment^{5, 6}.

1.5 Relevant Literature

CP is a disease of chronic pancreatic inflammation that is progressive with scarring and irreversible tissue damage resulting in loss of endocrine and exocrine function. Patients often have chronic abdominal and back pain, nausea and vomiting, weight loss, steatorrhea and increased number of bowel movements as the disease progresses. Diabetes may occur later in the course. Symptom severity is described as mild, moderate or severe and can change over time in individuals. Incidence is estimated to be from about 4.4 to 11.9 per 100,000 people per year⁷⁻¹⁴ and is more common in men. The prevalence of CP is estimated to be from about 37 to 42 per 100,000 people^{9, 11}. The approach to diagnostic testing and clinical care is not standardized, in part because there is no standard approach to diagnosis, diagnostic criteria vary, and many diagnostic approaches are costly and invasive⁴. Related to this protocol, there is little information on nutrition status and disease outcomes, and no clinical standards for screening or treatment of nutrition-related complications of CP. This proposal focuses on the specific issue of progressive loss of exocrine pancreatic function to the point of clinically meaningful malabsorption of dietary fat, and

concomitant loss of calories, fat soluble vitamins, minerals, and linoleic acid which are all essential nutrients for adult health. There is a large body of literature related to the diagnosis and treatment of CP, including some reports related to nutritional risks of malabsorption with CP. For example there have been a number of clinical trials using CFA to describe fat malabsorption in subjects with CP and confirmed pancreatic insufficiency and have demonstrated significant improvement in fat absorption from before to after pancreatic enzyme medication administration¹⁵⁻²¹. Ramesh et al¹⁵ found an increase in CFA% from 66.7±14.0 before pancreatic enzyme medication to 88.9±5.2% after pancreatic enzyme medication (a change of 22.7±12.2%) in Indian adults with CP. Also in adults from India participating in a placebo-controlled trial, Thorat et al¹⁶ saw CFA% increase from 66.5±14.1 to 86.1±7.5% in those taking pancreatic enzyme medication compared to an increase from 67.0±14.0 to 72.9±11.5% for placebo, a treatment effect of 13.7% (95% CI: 9.1, 18.2) after one week of pancreatic enzyme medication. Others have detected similar improvements in absorption in pancreatic enzyme medication trials in people with CP living in the US and in Eastern Europe^{17, 18}. Additional selected references from the past four years are noted here^{4, 22-43}.

Gene-environment interactions play a major role for the risk, development, and progression of pancreatic disease including both acute and chronic pancreatitis.⁴⁴ Major pancreatic risk factor genes have been identified (PRSS1, CTRC, CTFR, SPINK1),⁴⁴⁻⁴⁷ and others have been implicated (CaSR, CEL, CPA1, PRSS2-3, SBDS, UBR1 and CLDN2).^{44, 45} The association of specific genotypes with fat malabsorption in the context of CP remains to be fully explored.

Patients with CP are rarely evaluated for RPF/PI because there are no non-invasive, clinically available, affordable, acceptable and reliable diagnostic tests. Historically, the CFA is considered the gold standard for RPF/PI diagnosis. However, CFA is almost never used in clinical care in large part due to patient burden related to 72-hr stool and diet collections. The CFA method requires the patient consume a moderate fat diet, accurately document all food intake and collect all stool for 72-hours. A dietitian analyzes the record using software that calculates the daily and three day average fat intake (g/day). The multiple stool sample containers are shipped to one of a few laboratories that homogenizes and measures stool fat content (g/day). CFA% is calculated by the clinician once both diet and stool results are available. Lastly, reference ranges for healthy individuals evaluated with modern techniques for stool and dietary collections, and fat extraction and measurement are unavailable.

The MBT uses the general approach of the commonly utilized glucose tolerance test, with consumption of a beverage containing the nutrient of interest (fatty acid, rather than glucose) followed by the determination of the timing and amount of the fatty acids in blood samples. The MBT consists of administering two odd-chain length fatty acids: pentadecanoic acid (PA), a free fatty acid that does not require pancreatic lipase for absorption; and triheptadecanoic acid (THA), a triglyceride that requires pancreatic lipase for hydrolysis to three heptadecanoic (HA) fatty acids. These two fatty acids were selected for the MBT for the following reasons: 1) they are found in small amounts only in dairy foods; 2) the vast majority of individuals have very low serum concentrations of PA and HA; 3) they are safe even in larger doses than typically found in the diet; and 4) they are easily identified in the serum by well-established laboratory methods and absorption pharmacokinetics that can be readily described. Following the ingestion of the MBT fatty acids, the pharmacokinetics of the fat absorption for both PA and HA is assessed over an 8-hour period and the relative absorption of HA/PA is determined. A moment-based pharmacokinetic (PK) analysis is performed based on non-compartmental methodology using WinNonLin version 9.1 (Pharsight, Cary, NC). Baseline (C_0) and maximum (C_{max}) plasma concentrations are calculated, and area under the curve from time zero to eight hours (AUC) is calculated using the linear trapezoid

method. PK parameters are then compared between treatment groups using a paired t-test or Wilcoxon signed rank test as appropriate. To describe HA exposure relative to that of PA, the ratio of the HA to PA C_{max} (C_{max} HA/PA) and AUC (AUC HA/PA) is calculated for each subject after molar transformation and dose-normalization of exposure metrics. Also, population PK analyses for repeated-measures endpoints is conducted via nonlinear mixed-effects modeling with a qualified installation of the nonlinear mixed-effects modeling (NONMEM) software, Version VII, Level 2.0 (ICON Development Solutions, Hanover, MD)⁴⁸. Using this method, population PK modeling is conducted by simultaneously fitting structural PK models to both PA and HA concentrations.

We have shown that the MBT detects pancreatic-based fat malabsorption in healthy subjects using a lipase inhibitor medication (Orlistat)⁴⁹ and in subjects with CF and PI compared to healthy individuals and while on or off pancreatic enzyme medication treatment^{49,50}. Furthermore, the MBT can detect changes in fat absorption in subjects with CF and PI based upon the time of pancreatic enzyme administration related to food ingestion⁵⁰.

In this proposed study, we will use the MBT, CFA and BC methods to detect fat malabsorption in subjects with CP compared to healthy subjects, and the utility of the tests in detecting changes in fat absorption before and after pancreatic enzyme medication. The CFA is the “gold standard” for documenting fat malabsorption in research and in the regulatory environment. Currently CFA is the only method accepted by the FDA for evaluation of treatment of RPF/PI. Furthermore, a shorter sampling scheme for the MBT will be explored to optimize its potential use in clinical research. With these data from $n = 48$ participants including healthy comparisons and participants with varying severity of RPF/PI, pharmacokinetic methods will be employed to explore the possibility to reduce the time required and number of blood samples needed for the MBT to provide reliable classification of RPF/PI vs non-RPF/PI.

It is very likely that some patients with CP have RPF/PI and currently are not diagnosed or treated with pancreatic enzyme medication. There is no well accepted (non-invasive, acceptable patient burden, reasonably informative, and cost effective) approach to support clinical research or clinical care, nor are there established general clinical indicators or nutritional status risk factors to guide care of patients. Clinicians may avoid serious consideration of the possibility of exocrine pancreatic deterioration or just empirically try pancreatic enzyme medication. Improved care of patients with CP will be supported by additional well designed clinical research to evaluate approaches to diagnosis and establish risk factor profiles. The proposed study will contribute evidence to support future patient care in CP and physician education about RPF/PI and malnutrition in this group.

1.6 Compliance Statement

This study will be conducted in full accordance of all applicable Children’s Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent, and will report unanticipated problems involving risks to subjects or others in accordance with The Children’s Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

2.1 Primary Aims

2.1.1. Our first primary aim is to compare fat and energy absorption from the diet in subjects with CP and healthy subjects utilizing the MBT, CFA and BC methods to characterize fat and energy absorption patterns.

2.1.2. Our second primary aim is to determine if, in subjects with CP, fat and energy absorption will improve after pancreatic enzyme medication utilizing the MBT, CFA and BC methods to characterize the fat and energy absorption patterns.

2.2 Secondary Aims

2.2.1. The **secondary aim** of this proposal is to utilize these data to optimize the MBT design and application by reducing the time and blood samples required.

2.2.2. We will also **explore** the utility of the MBT compared to the CFA and BC (both require the burdensome 72-hour total stool and dietary intake collection) in identifying differences in fat absorption between subjects with CP and healthy individuals and within the CP cohort, with and without pancreatic enzyme medication.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

3.1.1 Screening Phase

Potential subjects will be screened using the protocol inclusion and exclusion criteria. Subjects will be recruited from regional academic hospitals and gastroenterology, and pancreatology subspecialty centers. Primary recruitment centers will include: 1) University of Pennsylvania Medical Center Hospital; 2) Pennsylvania Hospital; 3) Presbyterian Hospital; and 4) Philadelphia Veterans Administration Medical Center. Other likely collaborators and recruitment centers may include: 1) GI Centers at the Jefferson Medical Center; 2) Main Line Medical Center (Lankenau and Bryn Mawr Hospitals); and 3) other regional centers. These outside institutions will be involved with recruitment of subjects only and will otherwise not be engaged in the research. We will recruit the healthy subjects from the greater Philadelphia region using general adult practices, flyers, and recommendations from subjects with CP regarding healthy family and friends interested in participating. The CHOP Recruitment Enhancement Core (REC) will be utilized to recruit healthy adult subjects. The Recruitment Enhancement Core provides assistance with recruitment plan development and may assist in identifying and contacting potential participants using the Clinical Recruiting Unit (CRU), the CHOP Recruitment Registry and internal communication resources. Our research team anticipates no difficulty in enrolling well-qualified participants with the proposed sample size and staff (see **Table 2. Study Timeline**). Informed consent will be obtained prior to any study related procedures being performed, and this will include the discontinuation of pancreatic enzyme medication for those participants who are taking this medication at the time of enrollment in the study.

3.1.2 Study Treatment Phase

All study visits will occur at outpatient research offices of the Clinical and Translational Research Center at The Children's Hospital of Philadelphia (CHOP), a pediatric health care center. Eligible subjects will be enrolled into the study and come to CHOP for the study visits. Refer to Table 1 for list of assessments and the study timeline for the pace of recruitment.

3.1.3 Open-label treatment with Creon36™

Subjects with CP only will receive Creon36™ intervention and return to CHOP for Visit 2, about one to two weeks after their Visit 1. Refer to Table 1 for list of assessments and the study timeline for the visit schedule.

3.2 Allocation to Treatment Groups and Blinding

This is an open-label intervention therapeutic exploratory study, and not a randomized controlled trial.

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study Participation

The study duration per subject will be up to four weeks for the subjects with CP. The study duration for healthy subjects will be up to one week.

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at one investigative site in the United States, the Children's Hospital of Philadelphia. Recruitment will occur across regional centers as described above.

Recruitment will stop when 24 subjects with CP and 24 healthy comparison subjects are enrolled. It is expected that 24 subjects in each group will be enrolled to produce 22 evaluable subjects in each group.

3.4 Study Population

3.4.1 Inclusion Criteria

Chronic Pancreatitis Cohort

- Chronic pancreatitis diagnosis by gastroenterologist. Participants with CP will be characterized based on the TIGAR-O (toxic, genetic, autoimmune, recurrent, obstructive) etiology system, on pancreatic morphology (Cambridge criteria) when available, and on physiological state (exocrine and endocrine function) as recommended by the recent American Pancreatic Association Practice Guidelines⁴.
- Age 30-75 years old
- Evidence of at-risk for malabsorption including: 1) history of use of and response to pancreatic enzyme medication; 2) history of unintentional weight loss; 3) history of increased stools per week or fatty stools; and/or 4) other clinical signs or symptoms suggestive of fat malabsorption
- In usual state of health for past two weeks including no change in medications

-
- Able to consume a moderate fat diet for stool evaluations
 - Able to participate in the study for about four weeks with two study visits

Healthy Cohort

- Age 30-70 years old
- No known chronic disease that would affect dietary intake or fat absorption
- In usual state of health for past two weeks, with stable medications, diet and weight
- BMI from 14-35
- Able to consume a moderate fat diet for stool evaluations
- Able to participate in the study for about one week with one study visit

3.4.2 Exclusion Criteria**Chronic Pancreatitis Cohort**

- Evidence of normal fat absorption in medical record
- Medications that alter fat absorption (i.e. orlistat, other weight loss medications, ursodeoxycholic acid)
- Allergy to pork products
- History of intestinal blockage or fibrosing colonopathy
- Current diagnosis of gout and treatment with allopurinol medical therapy
- Pregnancy or breast feeding

Healthy Cohort

- Evidence of fat malabsorption
- Medications that alter fat absorption (i.e. orlistat, other weight loss medications, ursodeoxycholic acid)
- Pregnancy or breast feeding

4 STUDY PROCEDURES**4.1 Screening**

Subjects will be screened with support from the clinical sites using the protocol inclusion and exclusion criteria. Clinic staff will inform eligible individuals of the existence of this study and ask for permission from these individuals to share their contact information with the CHOP study staff. Either verbal or in person screening can occur. In the instance of screening over the phone, verbal subject permission will be obtained. In the instance of in person screening, study staff will obtain a signature from the subject. For both instances, screening consent will occur prior to obtaining any information from study subjects.

All subjects will be enrolled in their usual state of good health defined as no hospitalizations, emergency room or unscheduled acute illness clinic visits, and with activity levels and food intake considered typical by the subject for two weeks prior to Visit 1.

Healthy subjects will be identified from the greater Philadelphia region using referrals of friends and family of the subjects with CP. The subjects with CP will first ask for potential healthy subjects'

permission to release their contact information to the CHOP study team. Healthy subjects will also be identified using general adult practices, CHOP staff interested in participating in a research study, and through the CHOP Recruitment Enhancement Core (REC). The Recruitment Enhancement Core provides assistance with recruitment plan development and may assist in identifying and contacting potential participants using the Clinical Recruiting Unit (CRU), the CHOP Recruitment Registry and internal communication resources. Verbal subject permission will be obtained prior to obtaining any health information from these subjects to determine eligibility.

4.2 Study Treatment Phase

Subjects with CP will have two visits in this study. Visit 1 will be without pancreatic enzyme medication and Visit 2 will occur after they receive Creon36™ as their pancreatic enzyme medication therapy. Healthy subjects will have one visit only in this study. Both Visit 1 and 2 are identical with the exception of the several questionnaires (home environment, health history, quality of life) and fecal elastase assessment which are only collected once (refer to study procedures in Table 1). Collection of the saliva sample for genetic testing will only occur once, during Visit 1.

Visit for Consent and HIPAA Authorization for release of medical record information

- Informed Consent will be obtained at a visit to precede Day 1 of protocol before any study procedures take place. If subjects are taking pancreatic enzyme medication and will need to discontinue medication 3 days prior to Visit 1, the visit for informed consent will occur prior to the beginning of any discontinuation of the medication. HIPAA Authorization forms from the subjects' institutions will be obtained and will be signed in person or obtained by mail/fax/email.
- Instructions in preparation for study procedures will be given in person at this consent visit.

There are two protocols for Visit 1. The first protocol is for subjects with CP who are naïve to pancreatic enzyme medication, that is, they have not received pancreatic enzyme medication prior to Visit 1. The second protocol for Visit 1 is for subjects with CP who are currently taking pancreatic enzyme medication and will require a pancreatic enzyme medication washout period, that is, they will not take pancreatic enzyme medication for three days prior to Visit 1 (a "pancreatic enzyme medication washout" period). Subjects with CP will be prescribed Creon36™ as their pancreatic enzyme medication therapy and will receive this therapy for Visit 2. Visit 2 will be the same for all subjects with CP.

Healthy subjects will have Visit 1 only.

Visit 1: Protocol for All Healthy Subjects and for Subjects with CP who have not Received Pancreatic Enzyme Medication Prior to Visit 1

Day 1

- Regular lunch/dinner except for no intake of dairy products
- No alcohol intake
- Normal daily activity

-
- Fast overnight starting at 8 pm
-

Day 2 – CHOP Visit to CTRC Outpatient Lab and NGL

- Urine pregnancy test for pre-menopausal females
 - Insert heplock catheter
 - Baseline blood draw for MBT, Fat soluble vitamins (A, D, E, K), pre-albumin, serum zinc and selenium (Both Cohorts).
 - Baseline blood draw for CMP, CBC and fatty acid panel (CP Cohort only)
 - Saliva collection for DNA sequencing (CP Cohort only)
 - Administer MBT study meal (breakfast)
 - MBT – hourly blood sample for 8 hours
 - After hour 6 a low fat study lunch
 - Anthropometry (height, weight, skinfolds, circumferences)
 - Whole body DXA (CP cohort only)
 - Spot stool sample will be collected for Fecal Elastase I (CP Cohort only)
 - Questionnaires: Home Environment (Visit 1), Health History and medications, Quality of life questionnaire (MOS SF-36), PROMIS, Adherence, Adverse Events (Visit 2)
 - Instructions will be given for collection and shipping for: stool collection (72-hour fecal fat analysis and bomb calorimetry), 3-day weighed food records, and adverse events diary
 - Prescribe Creon36™ and dispense to subjects with CP (CP Cohort only)
-

Day 3 - Home

- Maintain adverse events diary
-

Day 4 - Home

- Moderate fat diet
 - 3-day weighed food record begins
 - Food record – Day 1
 - Maintain adverse events diary
-

Day 5 - Home

- Moderate fat diet
 - Food record – Day 2
 - Stool collection begins (72-hour fecal fat and bomb calorimetry), store frozen until brought to CHOP.
 - 72-hour stool – Day 1
 - Maintain adverse events diary
-

Day 6 - Home

-
- Moderate fat diet
 - Food record – Day 3
 - 72-hour stool – Day 2
 - Maintain adverse events diary
-

Day 7 - Home

- Moderate fat diet
- 72-hour stool – Day 3
- Maintain adverse events diary

Visit 1: Protocol for Subjects with CP who are Receiving Pancreatic Enzyme Medication Prior to Visit 1 and require a Washout Period

Participants discontinuing pancreatic enzyme medication will be without this medication for the nine days encompassing Visit 1.

Days 1 & 2

- Discontinue pancreatic enzyme medication (Washout)
-

Day 3

- Discontinue pancreatic enzyme medication (Washout)
 - Regular lunch/dinner except for no dairy intake
 - No alcohol intake
 - Normal daily activity
 - Fast overnight starting at 8pm
-

Day 4 – CHOP Visit to CTRC Outpatient Lab and NGL

- Urine pregnancy test for pre-menopausal females
- Insert heplock catheter
- Baseline blood draw for MBT, Fat soluble vitamins (A, D, E, K), pre-albumin, serum zinc and selenium (both cohorts)
- Baseline blood draw for CMP, CBC, and fatty acid panel (CP Cohort only)
- Saliva collection for DNA sequencing (CP Cohort only)
- Administer MBT study meal (breakfast)
- MBT – hourly blood sample for 8 hours
- After hour 6 a low fat study lunch
- Anthropometry (height, weight, skinfolds, circumferences)
- Whole body DXA (CP Cohort only)
- Spot stool sample will be collected for Fecal Elastase I (CP Cohort only)

-
- Questionnaires: Home Environment (Visit 1), Health History and medications, Quality of life questionnaire (MOS SF-36), PROMIS Survey, Adherence, Adverse Events (Visit 2)
 - Instructions will be given for collection and shipping for: stool collection (72-hour fecal fat analysis and bomb calorimetry), 3-day weighed food records, and adverse events diary
 - Prescribe Creon36™ and dispense to subjects with CP (CP Cohort only)
-

Day 5 - Home

- Maintain adverse events diary
-

Day 6 - Home

- Moderate fat diet
 - 3-day weighed food record begins
 - Food record – Day 1
 - Maintain adverse events diary
-

Day 7 - Home

- Moderate fat diet
 - Food record – Day 2
 - Stool collection begins (72-hour and bomb calorimetry), store frozen until brought to CHOP.
 - 72-hour stool – Day 1
 - Maintain adverse events diary
-

Day 8 - Home

- Moderate fat diet
 - Food record – Day 3
 - 72-hour stool – Day 2
 - Maintain adverse events diary
-

Day 9 - Home

- Moderate fat diet
 - 72-hour stool – Day 3
 - Maintain adverse events diary
-

4.3 Open-Label Treatment with Creon36™

Subjects with CP only will receive open-label treatment with Creon36™ as their pancreatic enzyme medication therapy and will receive this therapy for nine days encompassing Visit 2. Visit 2 will be the same for all subjects with CP.

Visit 2: Pancreatic Enzyme Medication Treatment Protocol for Subjects with CP Only

Days 1 & 2

- Pancreatic enzyme medication treatment (two Creon36™ with meals, and one Creon36™ with snacks)

Day 3

- Pancreatic enzyme medication treatment (two Creon36™ with meals, and one Creon36™ with snacks)
- Regular lunch/dinner except for no dairy intake
- No alcohol intake
- Normal daily activity
- Fast overnight starting at 8pm

Day 4 – CHOP Visit to CTRC Outpatient Lab and NGL

- Pancreatic enzyme medication treatment (two Creon36™ with meals, and one Creon36™ with snacks)
- Insert hepllock catheter
- Baseline blood draw for MBT, Fat soluble vitamins (A, D, E, K), pre-albumin, serum zinc and selenium, CMP, CBC and fatty acid panel
- Saliva sample for genetic testing
- Administer MBT study meal (breakfast)
- MBT – hourly blood sample for 8 hours
- After hour 6 a low fat study lunch
- Anthropometry (height, weight, skinfolds, circumferences)
- Adherence to pancreatic enzyme medication and Adverse Events Questionnaires
- Instructions will be given for collection and shipping for: stool collection (72-hour fecal fat analysis and bomb calorimetry), 3-day weighed food records, and adverse events diary

Day 5 - Home

- Pancreatic enzyme medication treatment (two Creon36™ with meals, and one Creon36™ with snacks)

-
- Resume usual diet
 - Maintain adverse events diary
-

Day 6 - Home

- Pancreatic enzyme medication treatment (two Creon36™ with meals, and one Creon36™ with snacks)
 - 3-day weighed food records begins
 - Food record – Day 1
 - Maintain adverse events diary
-

Day 7 - Home

- Pancreatic enzyme medication treatment (two Creon36™ with meals, and one Creon36™ with snacks)
 - Food record – Day 2
 - Stool collection (72-hour and bomb calorimetry), store frozen until brought to CHOP.
 - 72-hour stool – Day 1
 - Maintain adverse events diary
-

Day 8 - Home

- Pancreatic enzyme medication treatment (two Creon36™ with meals, and one Creon36™ with snacks)
 - Food record – Day 3
 - 72-hour stool – Day 2
 - Maintain adverse events diary
-

Day 9 - Home

- Pancreatic enzyme medication treatment (two Creon36™ with meals, and one Creon36™ with snacks)
 - 72-hour stool – Day 3
 - maintain adverse events diary
-

4.4 Unscheduled Visits

Due to the complexity of the study, no unscheduled visits will be permitted.

4.5 Subject Completion/Withdrawal

For subjects with CP who were naïve to pancreatic enzyme medication at the start of the study, at the end of participation in the study (all collections are complete for Visit 2) Creon36™ will be discontinued and they will resume their usual care without pancreatic enzyme medication. For subjects with CP who were receiving pancreatic enzyme medication prior to the start of the study,

at the end of participation in the study (all collections are complete for Visit 2), Creon36™ will be discontinued and they will resume their usual care and will resume the pancreatic enzyme product and dose they were receiving before the study began.

Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study treatment or visit schedules, or adverse events. The Investigator or the Sponsor may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents and on the CRF.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Medical Record Review

Variables that will be abstracted from the medical chart (paper or electronic):

- Date of birth - Age 30-75 years old
- Sex
- CP diagnosis: Participants with CP will be characterized based on the TIGAR-O (toxic, genetic, autoimmune, recurrent, obstructive) etiology system, on pancreatic morphology (Cambridge criteria) when available, and on physiological state (exocrine and endocrine function) as recommended by the recent American Pancreatic Association Practice Guidelines⁴.
- History of unintentional weight loss or history of increased stools per week
- Other clinical signs or symptoms suggestive of fat malabsorption
- History of pancreatic enzyme use
- History of allergy to pork, Current diagnosis of gout and treatment with allopurinol medical therapy, or intestinal blockage or fibrosing colonopathy
- All medications, particularly medications that alter fat absorption (weight loss medications or ursodeoxycholic acid)

5.1.2 Laboratory Evaluations

Serum 25-dihydroxyvitamin D and pre-albumin (vitamin A) will be assessed at CHOP laboratories. Serum retinol and α -tocopherol [vitamin E] will be assayed at Craft Laboratories. PIVKA II (Sarah Booth, Tuft's University, Medford, MA) and/or undercarboxylated osteocalcin % (UCOC %) will be assessed for vitamin K status (Caren Gundberg, Yale University, New Haven, CT). Zinc and selenium will be assessed at CHOP Laboratories using standard techniques. This will provide additional evidence for vitamin, mineral and essential fatty acid status resulting from pancreatic disease that may be improved with pancreatic enzyme medication in subjects with RPF/PI indicated by the MBT, CFA% and BC response. For subjects with CP, safety measures include results from serum comprehensive metabolic panel (CMP) and complete blood count with differential (CBC) performed at CHOP Laboratories using standard techniques. The CMP will provide serum albumin, total protein, bilirubin and liver enzymes to provide additional liver and nutritional status information for

the CP cohort. Also linoleic acid (essential fatty acid panel) will be assessed at ARUP Laboratories for the CP cohort only. All individuals from institutions outside of CHOP will receive only coded, not readily identifiable, samples for analyses and are not otherwise engaged in human subjects' research.

5.1.2.1 Pregnancy Testing

A urine pregnancy test will be performed for pre-menopausal female subjects.

5.1.3 Other Evaluations and Measures

Genetic Testing: Saliva will be collected for DNA sequencing of all exons and intronic junctions of all major pancreatitis risk factor genes, including PSSR1, CTSC, CFTR and SPINK1,⁴⁴⁻⁴⁷ in the CP group only. Results for additional genes that have been reported as CP disease associated will also be sequenced (CaSR, CEL, CPA1, PRSS2-3, SBDS, UBR1, and two SNPs near CLDN2). (Ariel Precision Medicine, Pittsburgh, PA). Saliva samples are stable long term (>2y) and can be stored at room temperature to ship in batches.

Fecal Elastase-1: Pancreatic function will be assessed for the CP group by fecal elastase-1 to determine the level of pancreatic enzyme activity in the stool^{51, 52} (Joli Diagnostics, Williamsville, NY). Subjects will be provided with the stool collection kit and proper instructions and supplies, and samples will be collected during the day of the visit if possible, or at home and returned. The stool sample will be stored at -20°C, and analyzed with a monoclonal enzyme-linked immunosorbent assay (ARUP Laboratory, Salt Lake City, UT).

Anthropometric Assessment: Weight, height, and BMI will be assessed. Measures of body composition will also be conducted for relative muscle and fat. All anthropometric techniques will follow those described by Lohman et al⁵³. Weight (0.1 kg) will be measured on a digital electronic scale (Seca, Munich, Germany) and stature (0.1 cm) on a stadiometer (Holtain, Crymych, UK). Skinfold thickness will be measured (0.1 mm) at the triceps, biceps, subscapular, and supra-iliac sites with a skinfold caliper (Holtain, Crymych, UK) to assess subcutaneous fat stores. Mid upper arm circumference measured with a non-stretchable fiberglass tape (0.1 cm) (McCoy, Maryland Heights, MO). Upper arm muscle and fat area Z scores will be generated^{54, 55}.

Body Composition: Total body composition, total FFM and FM and percent body fat (%FAT), will be assessed by the skinfolds using prediction equations adapted for children, adolescents, and adults^{56, 57}. Body composition will also be assessed by dual energy x-ray absorptiometry

(DXA) whole body scans (Delphi A, Hologic, Inc., Bedford, MA) for subjects with CP only at Visit 1. DXA uses very low-dose x-ray exposures (3 mrem) and measurements are rapid, making this a suitable technique for use in children. Standard positioning and clothing techniques are used. Quality control scans are performed daily using a simulated L1-L4 lumbar spine made of hydroxyapatite encased in epoxy resin, and a whole body composition phantom. A difference of >1.5% from the standard will be deemed out-of-range requiring servicing by the manufacturer. Whole body will be assessed by DXA at both Visit 1 and Visit 2. The scans will be analyzed to generate traditional DXA measures: whole body Area, BMC and areal-BMD (gm/cm²). In our institution, the in vitro CV is less than 0.6% and the in vivo coefficient of variation in adults is less than 1%. A urine pregnancy test will be performed on female subjects who are pre-menopausal prior to the scan. Pregnant subjects will not be scanned and will leave the study.

Dietary Intake: Three-day weighed food records will be obtained and calories, macro- and micro-nutrient content calculated. Subjects will be provided with scales, spoons and all supplies necessary for the collection of the dietary data and will be conducted with assistance from the CTRC staff and Bionutrition Unit^{58, 59}. Dietary intake will be analyzed using Nutrition Data System for Research software version 2012 developed by the National Coordinating Center (NCC, University of Minnesota, Minneapolis, MN)⁶⁰.

Disease Classification: Participants with CP will be characterized based on the TIGAR-O (toxic, genetic, autoimmune, recurrent, obstructive) etiology system, on pancreatic morphology (Cambridge criteria) when available, and on physiological state (exocrine and endocrine function) as recommended by the recent American Pancreatic Association Practice Guidelines⁴.

Home Environment and Health Questionnaire: The questionnaire will be administered via interview by the research staff, and will consist of two sections. The Health History section has general questions about the subject's health history including documentation of medical history, recent hospital admissions and illnesses, medication, history of pancreatic enzyme supplementation, and nutrient supplement use. A Home Environment section describes aspects of environment, such as education and income level, whether on Medicaid/Medicare and household size to describe the demographic characteristics. In addition to subjects' contact information (name, address, phone numbers); contact information from two additional contacts will be collected to maintain contact with the subject in the event that the subject cannot be contacted at their primary residence.

Quality of Life Questionnaires: In order to assess the health-related quality of life (QOL), validated questionnaires will be administered to all subjects at Visit 1. For all subjects, the Medical Outcomes Study 36-Item Short-Form Health Survey (MOS SF-36), a standardized, validated healthy survey will be administered. The MOS SF-36 covers physical functioning, bodily pain, general health perception, vitality, social functioning, emotional and mental health domains^{32, 61-65}.

Patient Reported Outcomes (PROMIS): We will assess patient reported outcomes at every study visit using the following NIH developed PROMIS short forms: pain, fatigue, depressive symptoms, physical function – mobility, physical function – upper extremity, and peer relationships.

Adherence: Adherence to pancreatic enzyme medication for subjects with CP will be assessed at study Visit 2 and by phone calls. This time will also be used to trouble-shoot any barriers to adherence, and also to collect information on adverse events experienced during the course of the study.

Adverse Events Diary: Subjects will be asked about all adverse events at both Visit 1 and Visit 2, and asked to rate by intensity (mild, moderate, severe). Serious adverse events will be reported as per various policies in a timely manner to CHOP IRB, and CTRC.

5.2 Efficacy

5.2.2. Diagnostic tests, Scales, Measures

Malabsorption Blood Test (MBT): The **MBT primary outcomes** consist of parameters from pharmacokinetic analyses. The MBT consists of a simultaneous oral dose of pentadecanoic acid (PA), a free fatty acid, and triheptadecanoic acid (THA), a triglyceride with three heptadecanoic (HA) saturated fatty acids requiring hydrolysis by pancreatic lipase before HA can be intestinally absorbed (Patent # US 7402405 B2, July 22, 2008). The MBT with the PA and THA is delivered in a breakfast test meal after a 12-hour fast and 24 hours without dairy foods. Serum concentration

levels of PA and HA are assessed by gas-liquid chromatography (GC), from serum samples drawn prior to MBT and then hourly for eight hours. The MBT test meal is prepared immediately before consumption, with 5.0g of PA and 5.5g of THA blended into a test meal composed of 64g vanilla Scandishake (Axcan Scandipharm, Birmingham, AL), 6oz silk light chocolate soy milk, and 10ml microlipids (www.nestle-nutrition.com). The 8oz MBT test meal contains ~550 calories, 32 g fat, and 52% of calories from fat. The test meal was designed to be similar to a high fat dinner meal. Prior to administration of the MBT test meal, an indwelling catheter is placed for baseline (0 Hour) and subsequent blood draws at Hour 1, 2, 3, 4, 5, 6, 7 and 8 post ingestion for quantification of serum PA and HA. The MBT test meal is consumed within five minutes of the baseline blood draw. After the Hour 6 blood draw, subjects consume a 1000 kcal, low fat (12 g) lunch meal, and two blood samples will follow this meal (Hours 7 and 8). During the eight-hour protocol period, subjects are offered non-caloric and non-caffeinated beverages *ad libitum*. The 72-hour home stool collection will begin two days after the MBT visit, and the corresponding 3-day weighed food record will begin the day prior to the first day of the 72-hour stool collection.

Table 3. Protocol for each MBT visit

Protocol	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Enzyme-naïve: MBT #1 CP Healthy Comparisons	- Fast from 8pm - No dairy - No alcohol - Normal activity	- MBT at 8am - Lunch at 2pm - 9 hourly blood draws	Home Return to usual diet	- Diet record (day 1)	- Diet record (day 2) - 72-hour stool (day 1)	- Diet record (day 3) - 72-hour stool (day 2)	- 72-hour stool (day 3)		
Enzyme-naïve with washout: MBT #1 CP	Wash-out No Enzymes	Wash-out No Enzymes	Wash-out No Enzymes - Fast from 8pm - No dairy - No alcohol - Normal activity	- MBT at 8am - Lunch at 2pm - 9 hourly blood draws	Home Return to usual diet	- Diet record (day 1)	- Diet record (day 2) - 72-hour stool (day 1)	- Diet record (day 3) - 72-hour stool (day 2)	- 72-hour stool (day 3)
Pancreatic Enzyme Medication: MBT#2 CP	Enzyme treatment	Enzyme treatment	Enzyme treatment - Fast from 8pm - No dairy - No alcohol - Normal activity	- MBT at 8am - Lunch at 2pm - 9 hourly blood draws	Home Return to usual diet	- Diet record (day 1)	- Diet record (day 2) - 72-hour stool (day 1)	- Diet record (day 3) - 72-hour stool (day 2)	- 72-hour stool (day 3)

Each subject in CP cohort will have two MBT, the first on a pancreatic enzyme medication-naïve protocol and the second after three days of pancreatic enzyme medication treatment. If subjects are receiving pancreatic enzyme medication at study enrollment, after consultation with their medical team, they will be asked to discontinue pancreatic enzyme medication for three days prior to the first MBT, the day of the MBT and the five days following the MBT until the end of the 72-hour stool collection. Thus, the total number of days of data collection for the enzyme-naïve MBT protocol will be seven for subjects not receiving pancreatic enzyme medication at Visit 1, and nine for those requiring a washout period from pancreatic enzyme medication (see **Table 3**). For the MBT that will be administered with pancreatic enzyme medication, subjects will take pancreatic enzyme medication for three days prior to the MBT. Each day, the pancreatic enzyme medication dose will be 72,000 lipase units per meal and 36,000 units per snack using Creon 36,000 units per capsule. They will take two Creon36™ (72,000 lipase units) with the MBT on the morning of the test meal. Pancreatic enzyme medication dose range for adults with RPF/PI is not well standardized.

The dose chosen for these short term studies are in the higher range recommended for initial treatment^{5,6}. Subjects will then continue to take pancreatic enzyme medication at home for the next five days until the 72-hour stool collection is completed. The total number of days for the pancreatic enzyme medication MBT protocol is nine (see **Table 3**). For the healthy individuals, the MBT protocol will last one week since they will be pancreatic enzyme medication -naïve.

Plasma samples will be analyzed by a GC method at Wake Forest University (Winston-Salem, NC). Total lipids are extracted from 200 μ L of plasma following the method of Bligh and Dyer⁶⁶. Following methylation of the fatty acids in the total lipid extract, fatty acid methyl esters are extracted into iso-octane and 1 μ L was injected into the GC column for analysis using a Hewlett Packard 5890 series II gas chromatograph with a programmable cool on-column capillary inlet, flame ionization detector (FID), and HP7673 auto sampler/injector. Chromatographic data collection and analysis is via a serial connection to a computer running ChromPerfect Spirit™ chromatography data system (Justice Laboratory Software, Denville, NJ). The column used is a CP-Sil 88 for FAME, 100m (L) x 0.25mm (ID) x 0.25 μ m (film thickness) (Agilent Technologies, Inc., Santa Clara, CA) with a 1m (L) x 0.53mm (ID) deactivated precolumn. Calibration curves are based on injections of methyl tridecanoate, methyl PA, and methyl HA injections. Inter-assay 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. Inter-assay variability for the measurement of HA (0.56, 1.29, 3.05 mg/dL) in the same samples is 2.6%, 4.0%, and 3.9%, respectively.

The primary outcomes for the MBT include PA and HA pharmacokinetic analyses including comparison of absorption curves, C_{max} and AUC, and percent absorption of plasma PA and HA concentrations and also the HA/PA ratio 1) between CP and the healthy reference group, and 2) with pancreatic enzyme medication exposure compared with pancreatic enzyme medication -naïve status in the CP cohort.

Utilizing the relative absorption of HA to PA, the MBT has been shown to respond to changes in fat absorption in healthy adult subjects using a lipase inhibitor and in subjects with CF and PI while on and off pancreatic enzyme therapy⁴⁹, and is also sensitive to enzyme dose titration⁵⁰.

Coefficient of Fat Absorption (CFA): The **CFA primary outcome**, the coefficient of fat absorption (CFA%) will be determined by the 72-hour stool collections at Visits 1 and 2 and a 3-day weighed food record collection while consuming a moderate fat (about 80+ g/day) diet. These stool collections will be performed at home and returned to CHOP. Subjects will be given a home collection kit and detailed instructions. Specimens will be stored frozen until analysis of total fat content by a gravimetric method (Mayo Medical Laboratories, Rochester, MN). Total dietary intake of fat during the 3-day period will be assessed from the 3-day weighed food records which will coincide with the 72-hour stool collection, and the CFA% will be calculated⁶⁷. The CFA% will be compared 1) between the CP cohort and the healthy reference group, and 2) with pancreatic enzyme medication exposure compared with pancreatic enzyme medication-naïve status in the CP cohort.

Bomb calorimetry (BC): The BC primary outcome for BC is the assessment of energy loss in the stool from the 72-hr stool collection (NIDDK sponsored research unit, Phoenix Indian Medical Center, Phoenix, AZ) using bomb calorimetry⁶⁸⁻⁷⁰. Samples will be shipped frozen and the heat of combustion (energy) will be measured in a bomb calorimeter, with energy liberated quantified in kcal/g stool. The energy loss from the stool will be compared 1) between the CP cohort and the

healthy comparison group, and 2) with pancreatic enzyme medication exposure compared with pancreatic enzyme medication -naive status in the CP cohort.

5.3 Safety Evaluation

Safety will be monitored by adverse events reporting. The frequencies of AEs by type, body system, severity and relationship to study supplement will be summarized. SAEs (if any) will be described in detail.

6 STATISTICAL CONSIDERATIONS

6.1 Primary Endpoint

The primary aim is to utilize the MBT, CFA and BC methods to characterize the fat and energy absorption patterns to compare fat and energy absorption from the diet in subjects with CP and healthy subjects. We will also determine if fat and energy absorption will improve after pancreatic enzyme medication administration in subjects with CP utilizing the MBT, CFA and BC methods to characterize the fat and energy absorption patterns.

6.2 Secondary Endpoint

The secondary aim is to utilize the data from the MBT, CFA and BC outcomes to optimize the MBT design and application by reducing the time and blood samples required. We will also explore the utility of the MBT compared to the CFA and BC (both require the burdensome 72-hour total stool and dietary intake collection) in identifying differences in fat absorption between subjects with CP and healthy individuals and within the CP cohort, with and without pancreatic enzyme medication. We will also explore the association of specific genotypes with fat malabsorption in the context of CP.

6.3 Statistical Methods

Analysis will begin with descriptive analyses of the study sample using means, standard deviations, medians, and ranges for continuous variables, and frequency distributions for categorical variables. Descriptive statistics and exploratory graphing such as frequencies, means, standard deviations, box plots, and scatter plots will be used to assess the normality of the data in terms of the presence of skew and/or outliers.

6.3.1 Efficacy Analysis

Statistical Analysis: H1 and H2: Descriptive statistics for MBT, CFA% and BC outcomes (mean, standard deviation, median, range, 95% CI) will be calculated for the CP cohort and for the healthy comparison group, and differences between CP and healthy groups assessed with unpaired t tests. For the MBT outcomes, a moment-based pharmacokinetic (PK) analysis will be performed based on non-compartmental methodology using WinNonLin version 9.1 (Pharsight, Cary, NC). Baseline (C_0) and maximum (C_{max}) plasma concentrations are calculated, and area under the curve from time zero to eight hours (AUC) is calculated using the linear trapezoid method. PK parameters can then be compared between treatment groups using a paired t-test or Wilcoxon signed rank test as appropriate. To describe HA exposure relative to that of PA, the ratio of the HA to PA C_{max} (C_{max} HA/PA) and AUC (AUC HA/PA) is calculated for each subject after molar transformation and dose-normalization of exposure metrics⁴⁹. Alternately, population PK analyses for repeated-measures endpoints can be conducted via nonlinear mixed-effects modeling with a qualified installation of the nonlinear mixed-effects modeling (NONMEM) software, Version VII, Level 2.0 (ICON

Development Solutions, Hanover, MD)⁴⁸. Using this method, population PK modeling is conducted by simultaneously fitting structural PK models to both PA and HA concentrations⁵⁰.

Descriptive statistics for MBT, CFA% and BC outcomes (mean, standard deviation, median, range, 95% CI) will also be calculated for the CP cohort with and without pancreatic enzyme medication administration, and the significance of changes over time assessed with paired t tests. HA and PA concentrations will be transformed to molar quantities and the ratios for HA to PA C_{max} and AUC and the associated 95% confidence interval will be calculated. A mixed effects modeling approach will be employed to examine the correlation of subject characteristics (age, BMI, sex, disease status, etc.) to PA and HA exposure metrics derived from the non-compartmental analysis to assess change over time in MBT response to pancreatic enzyme medication administration in subjects with CP. Metrics to be tested will be CFA%, PA and HA C_{max} , AUC, HA/PA C_{max} and AUC ratio and the %HA response compared to the healthy reference group. A value of $p < 0.05$ will be considered statistically significant.

H3: Pharmacokinetic modelling will be employed to identify the three to four blood samples over the first four to five hours of the MBT that provide the best and most reproducible estimate of the full eight-hour MBT (nine blood samples) in the CP cohort and healthy comparisons. The goal is a shorter sampling scheme for the MBT to optimize its use as a clinical diagnostic test.

In exploratory analyses, we will compare the estimates of percent fat absorption from the MBT with both the CFA% from the 72-hour fecal fat and diet analysis and the BC results (energy loss in the stool). We will compare the methods for detection of fat malabsorption as measured by CFA% and BC with fat absorption as measured by MBT for the subjects with CP compared to healthy individuals and also within the CP cohort before and after pancreatic enzyme medication use. A correlation analysis will be conducted to evaluate the performance of the MBT relative to that of the CFA%. HA and PA C_{max} , AUC, and HA/PA ratios will be plotted against CFA% or BC caloric loss to explore the relationship between the two tests. Any relationships observed in the plots will be further explored using correlation analysis and appropriate regression analysis as dictated by the observations. If a reasonable model can be developed to describe the response of MBT, CFA% and BC response to pancreatic enzyme medication treatment within the CP cohort, additional simulation studies will be performed to further examine the performance characteristics of both tests.

6.3.1 Safety Analysis

Safety will be monitored by adverse events reporting. The frequencies of AEs by type, body system, severity and relationship to study supplement will be summarized. SAEs (if any) will be described in detail.

6.4 Sample Size and Power

Sample Size Considerations: The primary hypotheses of this study: 1) the MBT, CFA and BC methods will detect differences in fat absorption between healthy subjects and subjects with CP when they are pancreatic enzyme medication -naive; and 2) the MBT, CFA and BC methods will detect changes in fat absorption with pancreatic enzyme medication administration in the CP cohort. For the MBT method, the sample size selected for the CP cohort ($n=24$) and the healthy comparisons ($n=24$) is based upon our previous experience in CF with detecting differences in C_{max} and AUC for HA and the HA/PA ratio⁴⁹, and in detecting the percent change in HA absorption (from pharmacokinetic modeling) compared to healthy subjects⁵⁰. For the CP vs. healthy comparison, 22 subjects in each group will have 80% power to detect a difference in means of 0.60 (the difference in C_{max} (in AUC)

HA/PA ratio between health group mean of 1.62 (1.30) and CP group mean of 1.02 (0.70)), which is 37% decrease in C_{\max} and 46% decrease in AUC from health group to CP group, assuming that the common SD is 0.68 using a t-test with $\alpha = 0.05$.

For the CFA% outcome, our experience has been in CF and PI, with average CFA% of $81 \pm 14\%$ in one study⁶⁷ and $83 \pm 10\%$ in another⁷¹ when subjects were on pancreatic enzyme medication. In comparison, in healthy subjects, CFA% is typically $\geq 93\%$. From the literature for CP, a number of clinical trials have used CFA% to describe fat malabsorption in subjects with CP and confirmed pancreatic insufficiency before and after administration of pancreatic enzyme medication. Ramesh et al¹⁵ found an increase in CFA% from 66.7 ± 14.0 before pancreatic enzyme medication to $88.9 \pm 5.2\%$ after pancreatic enzyme medication (change of $22.7 \pm 12.2\%$) in adults with CP from India. Also in adults from India participating in a placebo-controlled trial, Thorat et al¹⁶ saw CFA% increase from 66.5 ± 14.1 to $86.1 \pm 7.5\%$ in those taking pancreatic enzyme medication compared to an increase from 67.0 ± 14.0 to $72.9 \pm 11.5\%$ for placebo, a treatment effect of 13.7% (95% CI: 9.1, 18.2) after one week of pancreatic enzyme medication. From this combined experience with variability of the CFA% in both CF and CP, 21 subjects in each group will have 80% power to detect a difference in means of 10% (the difference in CFA% between health group mean of 95% and CP group mean of 85%) assuming that the common SD is 11% using a t-test with $\alpha = 0.05$.

For the BC method, there is limited information for typical variability in calories lost in stool in either healthy subjects or those with malabsorption diagnoses. Using 72-hour stool assessments, Wierdsma et al⁷⁰ has recently reported average daily energy loss of 213 ± 66 kcal in 23 healthy adults. This loss was less in those >50 years old (197 ± 49 kcal). If we assume that approximately 200 kcal/day are lost in the stool of healthy subjects, 21 subjects in each group will have 80% power to detect a difference in means of 50 kcal (the difference in BC between health group mean of 200 and CP group mean of 250 kcal lost in stool) assuming that the common SD is 56 using a t-test with $\alpha = 0.05$.

For the paired comparisons within the CP cohort before and after pancreatic enzyme medication use, 22 subjects will have 80% power to detect a difference in means of 0.44 (C_{\max} HA/PA ratio changes from 1.02 to 1.46 or AUC HA/PA ratio of 1.7 to 1.14), assuming a SD of differences of 0.68, using a paired t-test with $\alpha = 0.05$ two-sided significance level. We have demonstrated that differences of this magnitude in MBT outcomes (exposure metrics) indicating the degree of fat absorption between groups and with varying treatment regimens, are significant when samples of 16 subjects or less have been compared. We acknowledge that PA and HA absorption is moderately variable within subjects with CF and PI^{49, 50}. Between-observation variability was estimated to be 45.3% and 31.9% for PA and HA, respectively⁵⁰. For CFA%, from the literature on CP, the change in CFA% for subjects with CP and confirmed PI before and after pancreatic enzyme medication administration has ranged from 8 to 23% with the SD of that change ranging from 12 to 18%, but averaging at 14%. For CFA%, 22 subjects will have 80% power to detect a difference in means of 9% (increase in CFA% from 85 to 94% after pancreatic enzyme medication administration), assuming a SD of differences of 14%, using a paired t-test with $\alpha = 0.05$ two-sided significance level. For BC, 22 subjects will have 80% power to detect a difference in means of 36 (decrease from 250 to 214 kcal/day in the stool), assuming a SD of differences of 56, using a paired t-test with $\alpha = 0.05$ two-sided significance level.

We expect no more than 10% attrition in this study given a relatively short time period between first and second protocol visits. By enrolling 24 subjects in each group (CP and healthy), we can

account for attrition and also allow for the possibility of greater variability in the MBT, CFA% and BC outcomes within the CP cohort for which we have had no previous experience.

6.5 Interim Analysis

No interim analysis is planned.

7 STUDY MEDICATION

7.1 Description

CREON (pancrelipase) Delayed-Release Capsules 36,000 USP units of lipase (Creon36™), a pancreatic enzyme preparation, is a drug that requires prescription for use. It is approved for use, including the current indication and dosage, by the FDA.

7.1.1 Packaging

Creon36™ capsules containing 36,000 lipase units (LU) each will be provided (AbbVie, Inc.) to subjects with chronic pancreatitis. An 11 day supply of Creon36™ (one bottle or 100 capsules for approximately nine capsules/day) will be provided for nine days of use (81 capsules). Subjects will return unused capsules to the study team at the end of the study, and will be provided with mailing envelopes for this purpose.

7.1.2 Labeling

Storage will be at CHOP North Campus in room temperature (below 77° F) and dry conditions (humidity less than 70%). This facility is temperature controlled and continuously monitored.

7.1.3 Dosing

Participants will take two Creon36™ capsules (72,000 lipase units[LU]) at each meal for three meals a day, and one Creon36™ capsule (36,000 LU) with each snack up to three snacks a day for a total of nine capsules/day or 324,000 LU/day.

7.1.4 Treatment Compliance and Adherence

Adherence will be systematically assessed using the following methods: 1) One bottle of 100 capsules, enough for eleven days (approximately nine capsules/day) of Creon36™ will be prescribed for the subjects with CP at Visit 1 by Dr. Stallings, the Lead Investigator/ Study Physician investigator with experience in cystic fibrosis care and use of pancreatic enzyme medication, and dispensed to the subjects by the study team under Dr. Stallings' supervision. Participants will be asked to begin taking the Creon36™ capsules three days prior to their Visit 2 study visit to CHOP. 2) Subjects will be asked to complete an Adherence Survey, a semi-structured interview to document adherence to taking the Creon36™ over the nine days at Visit 2. We will maintain regular contact with participants via telephone and/or text message to ensure the subject has an adequate supply of Creon36™ and to obtain a cursory assessment of barriers to adherence so that we can develop individualized strategies to overcome these barriers. 3) Subjects will be provided with calendars on day 0 as a scheduling aid for study visits and a reminder to adhere to taking Creon36™. 4) Subjects will return unused Creon36™ capsules at the end of the study to the study team in a mailing envelope provided.

7.1.5 Drug Accountability

Records of Creon36™ receipt and disposition will be maintained by the study team. Records of receipts, and dispensing records will be examined during the course of the study. The purpose of these records is to ensure regulatory authorities that the Creon36™ will not be distributed to any person who is not a study subject under the terms and conditions set forth in this protocol. Creon36™ will be prescribed for the subjects with CP by Dr. Stallings, the Lead Investigator/ Study Physician, with experience in cystic fibrosis care and use of pancreatic enzyme medication, and dispensed to the subjects by the study team under Dr. Stallings' supervision, and may not be used for any purpose other than that described in this protocol. At study completion, all left over Creon36™ will be returned to the study team and this will be used as one estimate of adherence. Once the dataset is closed, leftover Creon36™ will be destroyed by the PI.

8 SAFETY MANAGEMENT

8.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study.

8.2 Adverse Event Reporting

Since the study procedures are not greater than minimal risk, SAEs are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) these will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has participated in the research protocol. The occurrence does not necessarily have to have a causal relationship with the research activities. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the research activities.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse experience occurring that results in any of the following outcomes:

- death
- a life-threatening event (at risk of death at the time of the event)
- requires inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect in the offspring of a subject

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.4.1 Relationship of SAE to investigation or other intervention

The relationship of each SAE to the study activities will be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

8.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the eIRB system and in accordance with the timeline below. External SAEs that are both unexpected and related to the study activities will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

8.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

8.6 Investigator Reporting of a Serious Adverse Event to Sponsor

Reporting will be consistent with regulatory and sponsor requirements. All serious adverse events experienced by a study subject receiving Creon36™ will be reported to the AbbVie, Inc. within 24 hours of learning of the event regardless of the relationship of the event to Creon36™, the AbbVie product. The PI shall make available to AbbVie promptly such records as may be necessary and pertinent to investigate any such event, if specifically requested by AbbVie. Medical Emergencies

For medical emergencies, we will follow the CHOP procedures for medical emergencies that may occur for adult visitors to CHOP.

9 STUDY ADMINISTRATION

9.1 Treatment Assignment Methods

This is an open-label intervention therapeutic exploratory study. All subjects with CP will be administered Creon36™. Healthy subjects will receive no intervention. This is not a randomized controlled trial.

9.2 Data Collection and Management

We will establish a database to store study data using standard software (e.g. RedCap). The database will be designed to perform automatic computations, such as exact age based upon birth date and date of exam, and averaging anthropometric measures, which are recorded in triplicate. Reports containing the number of subjects enrolled and data entered for each subject are generated and reviewed each month by the PI. The PI, or study staff, will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance. Following data entry, all primary and secondary endpoint data will be verified against original source documents. Data verification will be performed by someone other than the individual originally collecting and entering the data.

All subjects will be assigned a unique identification number that will be used to insure strict confidentiality. The databases are secured with password protection to insure confidentiality and security. The informatics manager receives only coded information which is entered into the database under those identification numbers. Electronic communication with outside collaborators involves only coded, not readily identifiable, information. A master list containing PHI and subject ID number will be kept separate from the data forms and the database that will only have a study ID number. The master list will be on a separate computer (password protected). Copies of the master list with PHI will also be stored on the CHOP secured server. All source documents including case report forms, laboratory results, and subject study binders will be kept in secured locations. The file cabinets and the study-specific room will be locked with access to study personnel only, and the outer hallway is also locked with limited access to CHOP research personnel.

Routine backup to the main study database, files created for analyses, and analysis programs will be completed. The main study database will be archived on a daily basis and stored on a CHOP secured server. The Informatics Core of the CTRC will create case report forms, set up the database in RedCap, and provide oversight for data entry and quality assurance for this study.

9.3 Confidentiality

Medical history information will be obtained at baseline. All of the materials collected are for research purposes only, and data will be kept in strict confidence. No information will be given to anyone without permission from the subject. This will be stated in the consent form.

Confidentiality is assured by use of identification codes. All data, whether generated in the laboratory or at the bedside, will be identified with an identification code unique to the subject.

To maintain confidentiality, private health information will be collected, accessed and stored in accordance with Institutional policies and HIPAA guidelines, including the use of multilevel password protection and enforcement of system user privileges on the database. To minimize the risk of disclosure, all direct identifiers will be removed as soon as possible and codes will be substituted for personal identifiers. PHI collected for this study will be kept up to five years after

final publication. Records of individuals are stored with ID numbers and a code with no discernible personal identifiers. Code lists and data files will be stored in separate, secure locations. Data will be accessed and stored on password-protected computers. To maintain confidentiality, codes will be used in the database, presentations and publications. The Investigator and other site personnel will not use such data and records for any purpose other than conducting the study.

9.4 Regulatory and Ethical Considerations

9.4.1 Data and Safety Monitoring Plan

The Principal Investigator (PI) (Dr. Zemel) is ultimately responsible for monitoring data integrity and patient safety and for overall study oversight. The study will be monitored weekly by the PI and the Lead Investigator (LI)/ Study Physician (Dr. Stallings). The study protocol will be carried out in accordance with OHRP and NIH guidelines and requirements. For participants with CP, AEs and SAEs that are unanticipated, serious, and possibly related to the study will be managed by the PI and Dr. Stallings (LI) LI/ Study Physician in consultation with the participant's adult gastroenterology physician team (Dr. Chandrasekhara (Perelman Center for Advanced Medicine/HUP [Penn Medicine/HUP]) or Dr. Rajala (Penn Medicine/ Philadelphia Veterans Affairs Medical Center [Penn Medicine/VA Hospital]) who will be immediately notified and will assume acute care management. Dr. Stallings will also consult with Dr. Mamula (CHOP/GI) who will be notified if an unexpected acute event occurs during a study visit at CHOP. For healthy participants, unexpected AEs and SAEs will be managed by the PI and Dr. Stallings (LI/ Study Physician) in consultation with the primary care physician identified by the participant upon enrollment in the study, who will be immediately notified. SAEs will also be reported to the IRB, CTRC, all members of the research team, the clinical care team, and sponsor in accordance with requirements. There will be ongoing collection of data on adverse events and compliance to the treatment protocol throughout the study by research staff and which will be reviewed weekly by the PI and LI / Study Physician (Dr. Stallings).

9.4.2 Risk Assessment

The most common side effects associated with taking the pancreatic enzyme Creon36™ in clinical trials were abdominal pain, hyperglycemia, hypoglycemia, frequent abnormal bowel movements, flatulence, vomiting, dizziness, sore throat and cough. Irritation of the inside of the mouth may occur if Creon36™ is not swallowed completely. Increase in blood uric acid levels (hyperuricemia), and severe allergic reactions (pork allergy) including trouble with breathing, skin rashes or swollen lips, although rare, may occur. Although it has never been reported, it is possible for a person to get a viral infection from taking pancreatic enzyme products that come from pigs. Fibrosing colonopathy and colonic strictures is a rare, serious adverse reaction associated with chronic high-dose pancreatic enzyme use over a prolonged period of time with enzyme doses in excess of 6,000 LU/kg body weight per meal or 10,000 LU/kg/day in patients with cystic fibrosis. The dose of Creon36™ prescribed for this study is a maximum of 324,000 LU/day. For an adult weighing 60 kg, this dose is 5,400 LU/kg/day or about half this amount. For an adult weight 40 kg, this daily dose is 8,100 LU/kg/day, still well below. An adult would have to weigh <33 kg (73 lbs.) to match or exceed the 10,000/kg/day dose that is considered excessive.

There are risks associated with discontinuing pancreatic enzyme medication for people with pancreatic insufficiency or reduced pancreatic function resulting in fat malabsorption. These include feelings of indigestion, stomach cramping after meals, gas, foul smelling, floating stools,

light-colored, orange or yellow stools, frequent or loose stools, or weight loss. Some participants in the study may be at increased risk for these symptoms.

- The participants receiving pancreatic enzyme medication prior to enrolling in the study who will discontinue use prior to the first study visit (Visit 1) may be at risk for signs and symptoms of fat malabsorption (described above) for nine days of the study. These nine days include the three days prior to study Visit 1 (washout period), the day of Visit 1 to CHOP, and the five days of specimen collection after Visit 1. These participants will resume their pancreatic enzyme product and dose for the period of time between the end of the nine days encompassing Visit 1 and the start of the three days prior to Visit 2 when they will switch to Creon 36™. Therefore, they will not be at increased risk for the signs and symptoms of fat malabsorption during the time between visits or during the nine days encompassing Visit 2 as they will be taking pancreatic enzyme medication throughout this period. When the study is completed, these participants will discontinue Creon36™ and resume their usual care and resume their usual pancreatic enzyme product and dose.
- The participants who are naïve to pancreatic enzyme medication prior to entering the study, will take Creon36™ for the nine days encompassing Visit 2 of the study, that is, three days prior to the protocol visit to CHOP, the day of the visit, and then five days after the visit until all specimen collections have been completed. If these participants have undiagnosed reduced pancreatic function and reduced fat absorption, they may have increased risk for the signs and symptoms of fat malabsorption upon discontinuation of Creon36™ at the end of the study. If their pancreatic function and fat absorption is normal, withdrawal from Creon36™ at the end of the study will not increase their risk for these symptoms.

The procedures in this study involve the potential risks related to the drawing of blood. The risks of drawing blood are rare, and minimal. There is a small risk of pain, infection and local irritation associated with insertion of an intravenous heplock catheter. However, this is considered a minimal risk and skilled research staff will insert the intravenous catheter. Each subject with CP will have approximately 100cc (approximately 6-7 Tbsp.) of blood drawn at each study visit. Each healthy subject will have approximately 88cc (approximately 5-6 Tbsp.) of blood drawn at their visit.

There is minimal risk associated with the low radiation exposure from the DXA scan. Radiation exposure is estimated to be no more than 3.0 μ Sv (depending on age and size) per visit, well below everyday background exposure (approximately 8.2 μ Sv). DXA instruments are regularly monitored for safety, and only experienced pediatric research personnel will conduct these tests.

There is minimal risk associated with collection of saliva for genetic testing. Potential risks related to genetic analyses can be to individuals or groups. These harms include stigmatization and insurability. To reduce this risk, no samples will be stored or used for future research. Information about this study will not be recorded in subjects' medical record. The genetic tests are being performed in a CLIA-certified lab, therefore results will be returned to subjects upon request. These results will not be included in the medical record. Any results that are clinically significant will be returned to the subject and included in their medical record. The likelihood of this happening is low.

Anthropometric measurements and pregnancy testing pose minimal risk to the subjects. There is minimal risk associated with sharing dietary intake, demographic information, health history and medical information.

Collection and storage of stool is associated with a small risk of fecal contamination. However, for safety and convenience, subjects will be provided with proper stool collection instructions and supplies (gloves, disposable collection containers, storage freezer container).

Private health information will be collected, accessed and stored according to HIPAA guidelines, including the use of multilevel password protection and enforcement of system user privileges on the database. To minimize the risk of disclosure, all direct identifiers will be removed as soon as possible and codes will be substituted for personal identifiers. Records of individuals are stored with ID numbers and a code with no discernible personal identifiers. Code lists and data files will be stored in separate, secure locations. Data will be accessed and stored on password-protected computers. To maintain confidentiality, codes will be used in the database, presentations and publications.

9.4.3 Potential Benefits of Study Participation

We cannot ensure a direct benefit to the subjects as a result of participating in this study.

Participants may benefit from knowing that they will contribute to a clinical research study that is important to the health of people with CP in the U.S. and around the world.

9.4.4 Risk-Benefit Assessment

The research we propose is justified, considering that the risk associated with participation is minimal compared to the potential and anticipated benefits. The benefits of participation clearly outweigh the risks, in view of the potential positive benefits of the study to the larger population of people living with CP.

9.5 Recruitment Strategy

It is expected that all subjects will be recruited by word of mouth and at the recommendation of the subjects' CP Care Team. Healthy subjects will be recruited using general adult practices, flyers, use of the CHOP Recruitment Enhancement Core (REC), and recommendations from subjects with CP of healthy family and friends interested in participating.

9.6 Informed Consent and HIPAA Authorization

Once a subject has expressed interest in participating a CHOP-based research team member will contact the subject via telephone and continue the introduction of the study to subjects. Verbal consent and HIPAA authorization for phone screening will be obtained prior to the collection of health information to determine eligibility. If screening occurs in person in the clinical care setting, participants will sign the screening consent prior to the collection of any health information to determine eligibility. A HIPAA Authorization form from the subjects' institutions for obtaining non-CHOP medical records, will be signed in person or obtained by mail/fax/email. All members of the team will be available (in person, by phone or email) to discuss the details and answer any study related questions as they arise. Once interest and eligibility are determined, procedures to set up enrollment will begin. At entry into the study, all subjects will be asked to review the study consent form. The Project Coordinator or other member of the clinical research team will meet with the subject to confirm the subject understands the study, and to answer any questions that the subject might have. A physician-level study team member will be available in person, by phone or by email

to answer any questions of issues that may arise. After all study-related questions are answered and subjects have had time to consider their decision, the Project Coordinator or member of the clinical research team will obtain fully informed, written consent from the adult subjects. The consent will be signed in the presence of a team member. Subjects will be given a printed copy of the signed, informed consent.

9.6.1 Waiver of Documentation of Consent/ Alteration of HIPAA Authorization

A waiver of documentation of consent will be sought for the verbal screening component of the study when potential participants are approached by phone. The rights and welfare of the subject will not be adversely affected because during the verbal consent process it is explained to the subject that we are recording their health information to determine eligibility for the study. The research could not practicably be carried out without this waiver as it is not practicable to approach and screen all potential participants in person. An alteration of HIPAA authorization will be sought for the screening component of the study. The use and disclosure of protected health information for the purposes of screening for eligibility involves no more than minimal risk to the privacy of participants, as the identifiers will be protected from improper use and disclosure, identifiers will be destroyed at the earliest opportunity consistent with the conduct of the study, and protected health information will not be reused or disclosed to any other person or entity, except as required by law for authorized oversight of the research project. The research could not practicably be conducted without access to and use of the protected health information collected during screening to determine eligibility.

Study staff stress during the verbal consent that the screening is voluntary, but necessary if they wish to participate, and that they can decline and stop the process at any point. Screening may also take place in person in which case the participant will sign the screening consent prior to the collection of health information to determine eligibility, and a waiver of documentation of consent will not apply. A written informed consent will be obtained prior to study entry and before any study procedures are performed, and this will include the discontinuation of pancreatic enzyme medication for those participants who are taking this medication at the time of enrollment in the study.

9.7 Payment to Subjects

9.7.1 Payments to subject

All participants are adults (30-75 yr.) and will be compensated \$200 for each visit for time and effort associated with each study visit: Visit 1 Visit 2. Subjects with CP will receive a total of \$400 (\$200 for each visit), while healthy subjects will receive \$200 (one visit only). Subjects living outside of the Philadelphia area will receive reimbursement for travel expenses (mileage, tolls, e.g.).

10 PUBLICATION

The research data obtained through the study outlined in this protocol will be shared with the research community, both through oral presentation at scientific meetings, and in written form, as published manuscripts. Reported factual material (primary data on which summary statistics and tables are based), commonly accepted in the scientific community as necessary to document and support research findings, will be provided in a timely fashion upon request by members of the scientific community to the principal investigator for a period of three years following acceptance for publication. The CHOP investigator will have access to the complete study data.

11 REFERENCES

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