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| **Protocol Title** | The Impact of Insulin Therapy on Protein Turnover in Pre-Diabetic Cystic Fibrosis Patients |
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| | Institutional Email Address: |
| **Scientific Assessment** | Nationally-based, federal funding organizations |
| **IND/IDE # (if applicable)** | N/A |
| **IND/IDE Holder** | N/A |
| **Investigational Drug Services # (if applicable)** | #4817 |
| **Version Number/ Date:** | Version 10/28 June 2021 |

**PROTOCOL COVER PAGE**

**REVISION HISTORY**

| Revision # | Version Date | Summary of Changes | Consent Change? |
|---|---|---|---|
| 6 | 11 June 2019 | Physical exam, visit windows, and safety levels added to the protocol, as well as minor administrative changes | Yes |
| 7 | 4 February 2020 | Would like to include a non-English speaking participant | Yes, added short form |
| 8 | 11 February 2020 | Spanish consent form and would like to add a remote consent process for the second parent if they are unable to make it. | Yes, added Spanish version |
| 9 | 6 July 2020 | Changing the inclusion criteria to say 24 months of having an abnormal OGTT. Due to COVID, the CF center is not doing routine OGTTs thus their results would not be within the 12 months. | No |
| 9.1 | 8 February 2021 | Adding an additional 12 control participants to be studied. | No |
| 10 | 28 June 2021 | Removing the CGM part of the protocol. We would like 30 controls in total. | Yes |
| | | | |

**Table of Contents**

**ABBREVIATIONS/DEFINITIONS**

- CF – Cystic Fibrosis
- CFRD – Cystic Fibrosis Related Diabetes
- CGM – Continuous Glucose Monitor
- BMI – Body Mass Index

## 1. Objectives

*1.1* Purpose: The current protocol describes a double-blind, placebo-controlled trial to determine whether insulin therapy improves protein catabolism in youth with CF and abnormal glucose tolerance, and to explore differences in efficacy between multiple daily pre-meal insulin dosing (as is currently standard for early CFRD) versus a more convenient once daily basal insulin dose (as has been used in small uncontrolled pilot studies). The findings of this study will provide a mechanistic rationale for instituting insulin in youth with CF and pre-diabetes, and will inform both research studies and clinical practice as to the best regimen for insulin delivery in this population.

Specific Aim 1

To conduct a double-blind, placebo-controlled study of the impact of daily insulin therapy on meal stimulated protein turnover in 60 youth age 10-25 years with CF and abnormal glucose tolerance. Assessment will occur before and after 1 month of insulin therapy.

*Hypothesis 1: In this insulin insufficient CF population, daily insulin replacement therapy will reduce meal stimulated protein catabolism from pre-insulin baseline, and will produce greater reductions from baseline than in placebo-treated patients.*

Specific Aim 2

To ascertain differences in the impact of 1X daily basal insulin versus 3X daily rapid-acting insulin on meal stimulated protein turnover in 60 youth age 10-25 years with CF and abnormal glucose tolerance.

*Hypothesis 2: A single daily dose of basal insulin will safely provide the same therapeutic benefit as the current standard method of insulin delivery of pre-meal 3x daily rapid acting insulin.*

Specific Aim 3

In a novel exploratory aim, we will assess the turnover of 3 individual proteins previously implicated in CF pathophysiology--- albumin, transferrin, and clusterin--- to determine the extent to which their synthesis and accumulation differs at baseline from normal controls and is subsequently affected by insulin therapy. Twenty matched healthy controls will be assessed at baseline only (no insulin therapy).

## 2. Background

*2.1.* Significance of Research Question/Purpose: Given the universal prevalence of insulin insufficiency in CF, the high lifetime risk of developing diabetes, the clinical impact of insulin insufficiency on protein catabolism and survival in CF, and the critical importance of maintaining body weight and LBM in this population, there is an urgent need to determine whether insulin replacement therapy should be instituted for anabolic purposes prior to the actual onset of diabetes and, if so, to ascertain the optimal regimen.

*2.2.* Preliminary Data: In a placebo-controlled clinical trial, insulin therapy improved body mass index (BMI) and LBM in patients with very early CFRD (CFRD without fasting hyperglycemia), and this is now standard care for these patients.

*2.3.* Existing Literature: N/A

## 3. Study Endpoints/Events/Outcomes

*3.1.* Primary Endpoint/Event/Outcome: Synthesis rates and relative accumulation of Individual proteins (albumin, transferrin, clusterin): the rate of plasma protein synthesis and breakdown can be measured using the amino acid isotopes infused during the isotopic meal study. Fractional synthesis rates of these proteins will be measured from the rate of incorporation of [13C6]Phe. We will purify these proteins by immunoaffinity chromatography and the isotopic enrichment in the respective proteins and plasma Phe will be measured as previously describe. The calculation of fractional synthesis rates of these proteins is as described previously using plasma [13C6]Phe as precursor. We will measure the isotopic enrichment in these proteins for 2 weeks after the isotope infusion is discontinued in order to monitor whether the isotope label is retained at a greater or lower rate as a measure of the accumulation of these proteins. If the isotope label is retained longer we will consider it an indication of lower turnover.

*3.2.* Secondary Endpoint(s)/Event(s)/Outcome(s):

Oxidative damage---In studies in type 1 diabetes, transient insulin deprivation caused accelerated plasma protein oxidative damage. We will assess the extent of oxidative damage by posttranslational modifications and deamidation in new and old isoforms of albumin, transferrin, clusterin, and other proteins. We will determine if insulin treatment prevents or reduces oxidative damage to specific proteins.

Protein Metabolomics—We will use ultra-performance liquid chromatography tandem mass spectrometry to measure 45 amino acids and their metabolites at weeks 0 and 6. It has been shown that amino acids (especially branched chain and aromatic amino acids) are sensitive predictors of developing diabetes in the future, and that branched chain amino acids, aromatic amino acids and several metabolites of amino acids such as citrulline and alpha-amino-adipoic acid are highly sensitive to insulin action. These amino acids and metabolites will be a secondary outcome to determine insulin effect.

## 4. Study Intervention(s)/Investigational Agent(s)

*4.1.* Description:

1. Subjects are admitted to the Clinical Research Unit here on the UMN campus in the early morning and 2 IVs are placed.

2. At ~6am subjects are given a priming dose of [15N]-Phe (0.7 mg/kg), followed by continuous infusion over the next 8 hours of 15[N]-Phe (0.7 mg/kg).

3. 2H5-Phe infusion begins at time 0 and continues for 5hr at a variable rate to mimic meal 13C6-Phe Ra.

4. During the second phase of the study, CF patients will have been receiving insulin (basal or bolus) or placebo for 1 month. They will receive their usual insulin/placebo dose during the protein turnover study.

5. To calculate protein dynamics, free amino acids are extracted from plasma using acetic acid and analyzed as their t-butyldimethylsilyl ester derivatives. The molar percent excess (MPE) of phenylalanine isotopes will then be calculated above background

*4.2.* Drug/Device Handling: Study drug, insulin or placebo (insulin diluent) will be obtained from the manufacturer in standard insulin vials. The investigational pharmacy will cover the label so that the study medication will be blinded.

- IDS #4817

## 5. Procedures Involved

*5.1.* Study Design: This double-blind, placebo-controlled trial of insulin therapy in CF youth with abnormal glucose tolerance will assess the impact of 2 different insulin regimens on protein turnover. In Phase 1, stable isotope measurement of meal-stimulated protein flux will be done in CF patients and normal controls, followed by bi-weekly fasting blood draws x2 wks to assess individual proteins. In Phase 2, CF patients will receive once daily basal insulin, 3X daily rapid-acting insulin, or injectable placebo for 6 weeks. After the 1st month, the meal-stimulated protein flux study will be repeated, followed by bi-weekly fasting blood draws x2 wks to assess individual proteins. Power analysis suggests 15 patients are needed per group; 20 are planned to account for drop-outs due to acute illness and non-adherence (although we expect good adherence since the treatment period is short).

*5.2.* Study Procedures:

Phase 1: Baseline Assessment, No insulin therapy, All CF patients and controls will complete this phase

Pre-Visit: CF patients will complete a dummy injection experience at the time of consent to see if they are okay with insulin injections. A collection of their latest physical examination will be grabbed from data records to make sure they are healthy. A physical exam, including tanner staging, can be completed at the pre-visit phase for CF patients.

Visit 0 (Baseline):

Meal-stimulated protein flux study (Jello Part): Control participants will be consented and a physical examination will be conducted by the PI at the beginning of this visit, CF patients have this done previously at a pre-visit. The study will start early in the morning (5am). They must have fasted since 8PM the night beforehand and encouraged to drink lots of water to help with IV placement. Two IVs will be placed, one for the isotope infusions and one for blood draws. After a 3 hour intravenous priming infusion, they will consume a jello test meal at time 0 and blood will be periodically sampled for the next 5 hours. The blood draws during the infusions are for cytokines, glucose, c-peptides, insulin, and isotopes. Participants that have CF and their parent/guardian will be asked to complete the Cystic Fibrosis Questionnaire-Revised, a standardized questionnaire created by the cystic fibrosis foundation, during the infusion. Also during the infusion part, a certified dietitian will come in and do a consult in regards to counting carbohydrates. The consult is necessary because it is important to know card counting when taking insulin. Once the infusion is completed we will give them a meal and take them over to get a DXA scan for determination of lean body mass. Visit 1 (week 1, +/- 1 day): outpatient blood draw to assess individual proteins

Visit 2 (week 2, +/- 1 day): For control patients they will complete an outpatient blood draw to assess individual proteins. End of study participation.

For CF patients: they will complete the same outpatient blood draw and start Phase 2.

Phase 2: Insulin therapy; All CF patients into 3 groups, No controls

Randomized into group (once daily basal insulin, 3x daily rapid acting insulin, or placebo) Insulin (basal or rapid) or placebo, will be supplied by the manufacturer in identical standard insulin vials. Participants will be instructed in subcutaneous injection technique. Study medication adjustments will be made under the supervision of the research teams, who are quite familiar with insulin management in patients with CF, and who will be available at all times for support.

Visit 3 (Week 6, +/- 7 days): Complete the meal-stimulated protein flux study part again from Phase 1 along with the DXA scan, CFQ-R, and CGM placement. There will not be a dietitian consult at this visit.

Visit 4 (Week 7, +/- 1 day): outpatient blood draw to assess individual proteins and removal of continuous glucose monitor

Visit 5 (Week 8, +/- 1 day): outpatient blood draw to assess individual proteins and end study.

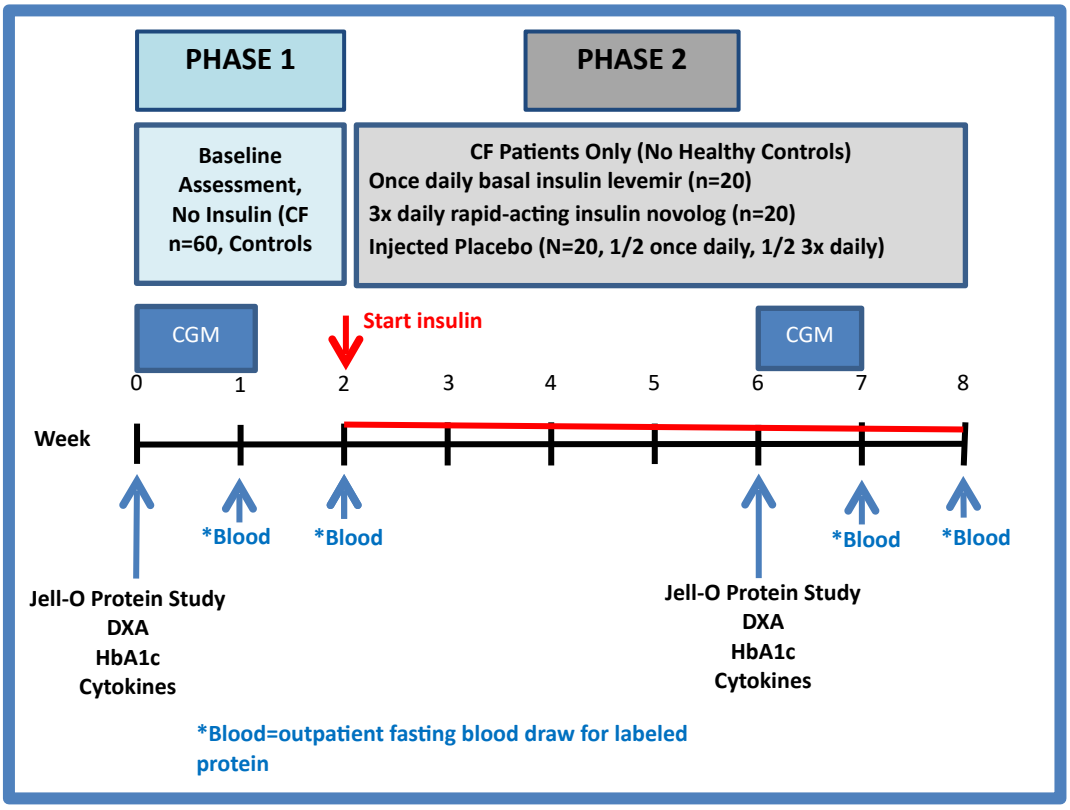
Between Visits:

Study personnel will contact (e.g. telephone, email) patients every three days while insulin is being adjusted and weekly thereafter in addition to their study visits. During these contacts staff will review and adjust (if needed) the medication dose, review hypoglycemia symptoms and management, monitor acute illness or adverse events, provide education, and encourage adherence.

Participants will also be asked record their blood glucose levels at random times during the entire study. These need to be recorded to determine clinically if insulin adjustment is necessary.

Patients will receive all usual CF care; study participation will not impact standard-of-care treatment.

*5.3.* Study Duration: Each study participant will be in the study for an 8 week duration.




- Anticipated duration to recruit all study participants is 4.5 years.
- The anticipated duration to complete all study procedures, including any long-term follow-up, and data analysis is 5 years.

*5.4.* Individually Identifiable Health Information: Research participants in this study will sign an Authorization to Use or Disclose Protected Health Information for Research Purposes.

*5.5.* Use of radiation*:* See attached UHS application

## 6. Data and Specimen Banking

N/A

## 7. Sharing of Results with Participants

*7.1.* After all subjects have completed the study and the results are analyzed (about 5 years total), the PI will let the subjects know the overall study results and whether they received insulin or placebo.

## 8. Study Population

*8.1.* Inclusion Criteria:

1. Diagnosis of cystic fibrosis, age 10-25 years

2. A standard routine annual OGTT performed within 24 months of randomization

3. Abnormal glucose tolerance, with a fasting glucose level <126 mg/dl and

a. The 1-hr OGTT glucose is ≥200 mg/dl but the 2-hr glucose is <140 (INDET), OR

b. The 2-hour OGTT glucose is 140-199 mg/dl (impaired glucose tolerance, IGT).

*8.2.* Exclusion Criteria:

1. Diagnosis of CFRD, Consensus Conference definition

2. Previous organ transplant, or transplant imminent during study period

3. BMI percentile >95

4. Treatment with systemic glucocorticoids (nasal or inhaled glucocorticoids are acceptable)

5. Therapy with growth hormone or Megace

6. Nighttime continuous drip gastrostomy/jejunostomy feedings

7. Pregnancy or breast-feeding or plans to become pregnant during study period

8. Any change in medications during the 3 months prior to the study

- Exception 1: Any antibiotics given for an acute illness greater than 6 week prior to study start. Antibiotic therapy is a routine treatment plan for acute illnesses in this population and is not a relevant medication therapy change for this study.

- Exception 2: the new corrector/potentiator combination drug lumacaftor/ivacaftor is expected to get FDA approval in early 2015, and most CF patients with severe genotypes, including many eligible for this proposal, will receive this drug. This is not a contraindication to participation in the current proposal (and participation in other studies is not contraindicated in the PROSPECT post-marketing drug study). Though the primary effects of the combination therapy appear to be apparent after 1 month, we will wait 6 months after initiation of lumacaftor/ivacaftor before enrollment in this study to make sure subjects are in a steady state.

9. Any anticipated change in medication during the 3 month study period

10. Acute illness in the 6 weeks prior to enrollment

*8.3.* Screening: Screening begins with identification of eligible subjects using the standard OGTT recommended annually for all CF patients. Currently at UMN we have 121 CF patients age 10-25, 58 of whom have IGT or INDET and meet study entrance criteria. Nine new patients per year in this age range are diagnosed with IGT or INDET at UM and thus become eligible for the study. An additional 46 non-diabetic children and youth age 10-25 years are followed at our Children's Hospitals and Clinics of Minnesota CF affiliate, 20 of whom currently have abnormal glucose tolerance. Three to four new patients per year become eligible. Our experience from previous CF studies in this age group suggests ~60% will agree to participate. Over the study period we anticipate that 45-50 CF patients will participate from UM and 10-15 from Children's. Normal control subjects will be recruited from fliers placed in the clinics and the University campus. In addition, siblings of CF participants will be invited to participate as controls. CF subjects will be randomized in a 1:1:1 ratio to basal insulin, pre-meal insulin, or injectable placebo (half 3x/daily pre-meal, half 1x/daily), stratified by 2 age intervals (10-17, 18-25y) and gender. Randomization will be done with computer-generated lists for each strata, in randomly alternating blocks of 3 and 6. The research subject, investigator and clinic personnel will be blinded to assignment. Healthy controls will be matched for gender, age and BMI percentile. The statistician will review control recruitment after every 5 subjects to ensure they are representative of the patient groups and determine whether specific control ages or genders should be recruited.

*8.4.* Vulnerable Populations:

☒ Children
☐ Pregnant women/Fetuses/Neonates
☐ Prisoners
☐ Adults lacking capacity to consent and/or adults with diminished capacity to consent, including, but not limited to, those with acute medical conditions, psychiatric disorders, neurologic disorders, developmental disorders, and behavioral disorders
☐ Approached for participation in research during a stressful situation such as emergency room setting, childbirth (labor), etc.
☐ Disadvantaged in the distribution of social goods and services such as income, housing, or healthcare
☐ Serious health condition for which there are no satisfactory standard treatments
☐ Fear of negative consequences for not participating in the research (e.g. institutionalization, deportation, disclosure of stigmatizing behavior)

☐ Any other circumstance/dynamic that could increase vulnerability to coercion or exploitation that might influence consent to research or decision to continue in research
☐ Undervalued or disenfranchised social group
☐ Members of the military
☒ Non-English speakers
☐ Those unable to read (illiterate)
☐ Employees of the researcher
☐ Students of the researcher
☐ None of the above

*8.5.* Additional Safeguards:

Given the universal prevalence of insulin insufficiency in CF, the high lifetime risk of developing diabetes, the clinical impact of insulin insufficiency on protein catabolism and survival in CF, and the critical importance of maintaining body weight and LBM in this population, there is an urgent need to determine whether insulin replacement therapy should be instituted for anabolic purposes prior to the actual onset of diabetes and, if so, to ascertain the optimal regimen. The current protocol describes a double-blind, placebo-controlled trial to determine whether insulin therapy improves protein catabolism in youth with CF and abnormal glucose tolerance, and to explore differences in efficacy between multiple daily pre-meal insulin dosing (as is currently standard for early CFRD) versus a more convenient once daily basal insulin dose (as has been used in small uncontrolled pilot studies). The findings of this study will provide a mechanistic rationale for instituting insulin in youth with CF and pre-diabetes, and will inform both research studies and clinical practice as to the best regimen for insulin delivery in this population.

Both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.

The minor will be able to give assent and if he/she chooses not to participate, the choice will be honored.

## 9. Local Number of Participants

*9.1.* Local Number of Participants to be Consented: We have consented 40 CF patients, and need 30 control patients in total.

## 10. Local Recruitment Methods

*10.1.* Recruitment Process: Normal control subjects will be recruited by flyers located around the University campus and word of mouth. CF patients who

have had abnormal glucose tolerance documented in the last 24 months will be contacted by Dr. Moran, who will describe the research and the fact that it is voluntary, and determine if patients are interested and eligible. Dr. Moran maintains the CF OGTT Database and follows these patients clinically.

*10.2.* Identification of Potential Participants: Dr. Moran or members of her research team will make initial contact with CF patients. Normal controls will contact research staff- contact information will be provided on advertisements around campus. Dr. Moran will confirm with the CF office that the patient has signed an agreement to release their PHI contained in their medical records and to be contacted for research purposes. Only a handful of the more than 500 CF patients at the UM CF Center have not provided such consent.

*10.3.* Recruitment Materials: A recruitment flyer will be used for Healthy Controls. A phone call, an email, or face-to-face contact may be used by the PI for potential CF subjects.

*10.4.* Payment: CF patients: max $400;

$140 per jello meal study x2

$15 per blood draw x4

$60 for bonus completion

Healthy controls: max $200

$140 per the clamp

$15 per blood draw x2

$30 bonus for completion

Study subjects will be receiving the compensation that will be mailed within 6 weeks of each of their visits.

## 11. Withdrawal of Participants

*11.1.* Withdrawal Circumstances: If the subject becomes ill or hospitalized during the study period, the PI will inform them that they will be withdrawn.

*11.2.* Withdrawal Procedures: All data collected up to the point of withdrawal will be kept by the investigator. The investigator will let the subject know that they might be contacted in the future to possibly restart the study.

## 12. Risks to Participants

*12.1.* Foreseeable Risks:

1. The CF patients who will receive insulin and they are already insulin insuffcientand have abnormal glucose tolerance. Hypoglecemia is always a risk with

insulintherapy. They could also have hypoglemic reactions during the jello part of the study. This will be closely monitored and they will receive juice if symptoms arise.

2. There is potential for discomfort as the iv is being inserted and at the iv site.

3. The stable isotopes to be infused are naturally occurring isotope of amino acids and thus the amino acids are not anticipated to pose any risk. During preparation of any iv solution there is the potential for contamination which is why the isotopes will be prepared by the investigational pharmacy under their usual sterile protocol.

4. Confidentiality may be breached.

5. Nausea from eating the Jello.

6. DXA is an x-ray that has very little radiation exposure.

7. The continuous glucose monitor may cause some discomfort, inflammation or irritation at the site.

## 13. Potential Benefits to Participants

*13.1.* Potential Benefits: It is possible that there is no benefit for participants because they may not receive active drug. There is a possibility that insulin replacement therapy may be effective in breaking the cycle of underweight and overall clinical deterioration because of its potent anabolic effects on protein turnover.

## 14. Statistical Considerations

This study will compare the three parallel treatment groups (placebo, basal insulin, and pre-meal insulin) on the basis of within-subject changes from week 0 (baseline) to week 8 (after 1 month of assigned treatment). The primary endpoints are within-subject differences from week 0 to week 8 in protein turnover parameters, change in Phe flux (ΔQP) and in conversion of Phe to Tyr (ΔQPT). Treatment groups will be compared by pairwise contrasts in analysis of variance, adjusted for baseline protein turnover. The primary-endpoint analysis will be per-protocol, performed on adherent subjects who received at least 70% of study medication in order to assess insulin effects. In addition, as part of planning for a larger trial, an intent-to-treat analysis will be performed, including all randomized participants to assess insulin treatment effects in practice. Exploratory analyses will examine associations between differences in protein turnover parameters and mechanistic endpoints: baseline pulmonary function, BMI percentile, dietary intake (from diet records), observed plasma glucose mean and MAGE (from CGM), cytokines, and treatment adherence.

Analysis of secondary protein turnover endpoints will be exploratory extensions of previous work (37, 38, 57, 58). For the 3 proteins of interest (albumin, transferrin, clusterin), both the fractional synthesis rate and relative isotopic enrichment (ratio of isotopic enrichment to the baseline enrichment) will be calculated from each of the 4 weekly blood samples after both the week 0 and week 8 labeled-infusion studies. At these same points we will also assess the accumulation of damaged proteins, since

oxidative stress is likely to be reduced during insulin therapy. The baseline (wks 0-4) comparison will be between CF participants and healthy controls, while the comparison of the treatments will use measurements from both weeks 0-4 and 8-12. The profiles of these rate changes over time will be compared visually, by summary measures such as slope if applicable, and by mixed effects linear model for longitudinal trends, with participants treated as a random effect to model the correlation between repeated observations from the same subject.

Protein metabolomics will be assessed during the in-patient studies at week 0 and week 8. The baseline comparison will be between CF participants and healthy controls, while the comparison of the treatments will use changes from week 0 to week 8. The comparison of protein metabolomics will use principal components analysis of the baseline protein and metabolite measurements to find separated clusters of CF participants versus healthy controls, and principal components analysis of the week 8 measurements to compare clusters between the three treatment groups.

Secondary mechanistic endpoints are measured weekly during both baseline and intervention phases: 3-point glucose profiles, mean and MAGE plasma glucose will be measured at weeks 1-12 using CGM. We will use a mixed-effects linear model to compare these longitudinal profiles of plasma glucose between treatments, adjusting for the appropriate baseline value and treating participants as a random effect to model the correlation between repeated observations from the same participant.

The safety analysis will estimate and compare rates of hypoglycemia and subclinical hypoglycemia (identified form CGM) between treatments using Poisson regression. In addition, rates of non-adherence will be compared between treatments.

**Power Analysis**

We anticipate that 45 participants, 15 from each of 3 arms, will provide sufficient power to detect clinically significant improvements in protein turnover (ΔQP and ΔQPT) at baseline, based on comparisons between healthy controls and insulin-insufficient CF patients in our previous study (18). Details are given below. We have inflated the sample size to 20 per arm to allow replacement of drop-outs and non-adherent participants, participants who develop diabetes, or suffer an acute exacerbation. The most common reason for drop out is likely to be acute illness, although the brief (3 month) time span will help reduce this. Also, given the short time span of this study we anticipate adherence will be good. This is in line with other studies in CF where about 80% of subjects are adherent at least 80% of the time (24, 65). Because many of our subjects will be adolescents, one might expect somewhat worse adherence. However, we believe this will be offset by the short duration of the study and by mechanisms to encourage adherence such as school nurse supervision on school days and frequent contact with study personnel. Study drop-out may occur due to development of fasting hyperglycemia or acute illness. These are discussed in greater detail in the human

subjects section. We expect less than 2% of subjects to develop CFRD during the 3 month study period. Patients who experience acute illness during the study may repeat it when they are well. Thus, we have inflated our group size to account for 20% non-adherence, 1-2% development of diabetes, and 11-12% acute illness.

Because studies of the effect of chronic insulin therapy have not been conducted previously in CF patients, precise estimates of treatment effect cannot be made. We previously demonstrated significant insulin treatment effects protein on *de novo* synthesis in 14 non-CF study participants, 7 with type 1 diabetes and 7 without (66). Previous work (24) gave estimates for CF patients of the standard deviation (SD) of changes in protein turnover parameters ΔQP and ΔQPT, differences between conditions of insulin infusion and baseline fasting. The primary endpoint is the within-subject change in ΔQP and ΔQPT from week 0 (baseline) to week 8 (after 1 month of assigned treatment), and we estimated the SD of these changes as (SD of ΔQX)$\sqrt{2(1-\rho)}$, assuming ρ=.6 for within-subject correlation, for ΔQP = 4.0 and ΔQPT = 1.26. The table below reports these standard deviations and shows corresponding minimum differences that the study would have 80% power to detect with sample sizes of 15 or 20 in each group. For context, these minimum detectable differences are also given as percent of the baseline value in the CF patients. Observed mean differences in ΔQP and ΔQPT between CF patients and healthy controls were *larger* than the minimum detectable differences for n = 15/group (24). To the degree that either insulin treatment is able to normalize protein turnover in the CF patients, these observed differences suggest possible treatment effects.

| N per group | Minimum detectable | Minimum detectable difference as % of | Observed mean difference (CF patients – |
|---|---|---|---|
| **ΔQP** (SD = 3.6) | | baseline mean = 50.8 | 6.0 |
| 15 | 3.8 | 7% | |
| 20 | 3.3 | 6% | |
| **ΔQPT** (SD = | | baseline mean = 6.43 | 1.5 |
| 15 | 1.2 | 19% | |
| 20 | 1.0 | 16% | |

The unblinded study statistician will perform new sample size calculations after the first 15 CF subjects (5 in each group), and, if necessary, additional subjects will be studied.

## 15. Confidentiality

*15.1.* Data Security: The PI, Dr. Moran, will store and maintain data indefinitely on her password protected UM desktop computer in her locked office which is maintained by the AHC.

## 16. Provisions to Monitor the Data to Ensure the Safety of Participants

*16.1.* Data Integrity Monitoring.

The protein turnover portion of the study will only be done at the University of Minnesota where the staff have the necessary expertise to perform these complicated medical studies. Dr. Moran is in regular (at least monthly) communication with Dr. Larson Ode at the University of Iowa to ensure all other study procedures are handled in a consistent fashion. In order to ensure the validity of subject characteristics that may impact the rigor of the study, we will continue to work to make sure we have approximately equal gender distribution and that we have a similar number of pediatric (age 10-18) and young adult (age 19-15) subjects. In order to ensure robust and unbiased results, we are working with collaborators in Italy who are considered the world experts in metabolic mathematical modeling. We send them the raw mass spectroscopy data and they perform the mathematical analysis. They are blinded to treatment arm, which will help ensure the validity of the data.

*16.2.* Data Safety Monitoring.

In addition to the two study visits, the study nurse coordinator is in weekly contact with study participants and all adverse events are recorded on case report forms. The safety data including all adverse events are reviewed monthly by the investigators. The only significant potential risk directly associated with this study is that of severe hypoglycemia, if the insulin dose is too high for the subjects. In fact, we have now studied 24 subjects and have not had one single episode of severe hypoglycemia, so the risk is low. The only adverse events that have occurred are respiratory infections as is expected in cystic fibrosis. The investigators are all pediatric endocrinologists, well experienced with insulin administration and management of hypoglycemia. Efficacy data will not be reviewed until study end. No conditions have been identified that would trigger an immediate suspension of the research.

## 17. Provisions to Protect the Privacy Interests of Participants

*17.1.* Protecting Privacy: Your records will be kept private. Dr. Moran will keep the study data and your name on her password-protected desktop computer kept in her secure UM office. No one outside of Dr. Moran and the research team will have access to your name or identifying information. All samples sent to the Mayo Clinic will not have any information from which you could be identified. In any publications or presentations, we will not include any information that will make it possible to identify you as a

subject. Your record for the study may, however, be reviewed by departments at the University with appropriate regulatory oversight. To these extents, confidentiality is not absolute.

*17.2.* Access to Participants*:* Research staff may have to look through medical records if an enrolled subject becomes hospitalized or starts any new medication. This is a way that the research staff can document what is happening and know how to proceed.

## 18. Compensation for Research-Related Injury

*18.1.* Compensation for Research-Related Injury: In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to you or your insurance company.

*18.2.* Contract Language: In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner to you or your insurance company. If you think that you have suffered a research related injury, let the study physicians know right away.

## 19. Consent Process

*19.1.* Consent Process (when consent will be obtained):

- Dr. Moran will have the original discussion with participants over the phone or face-to-face in clinic and will then send/give a copy of the consent to read. She will then follow up with them in person or on the phone to assess their interest level and answer any questions. Interested patients will then be consented in clinic by Dr. Moran and given a demonstration of the dummy injection for insulin. This pre-visit will occur within 3 months of the baseline visit.
- What is the purpose of this study?
- What if you decide you don't want to do this study anymore?
- What are the side effects?
- What is the benefit to you?
- It will be made clear during recruitment and informed consent process that this is a voluntary study. Each time the participant is scheduled, they will be made aware again that their participation

is voluntary and no relations will be changed if you decide you no longer want to participate.

*19.2.* Participants Who Are Not Yet Adults (infants, children, teenagers under 18 years of age):

- Given the universal prevalence of insulin insufficiency in CF, the high lifetime risk of developing diabetes, the clinical impact of insulin insufficiency on protein catabolism and survival in CF, and the critical importance of maintaining body weight and LBM in this population, there is an urgent need to determine whether insulin replacement therapy should be instituted for anabolic purposes prior to the actual onset of diabetes and, if so, to ascertain the optimal regimen. The current protocol describes a double-blind, placebo-controlled trial to determine whether insulin therapy improves protein catabolism in youth with CF and abnormal glucose tolerance, and to explore differences in efficacy between multiple daily pre-meal insulin dosing (as is currently standard for early CFRD) versus a more convenient once daily basal insulin dose (as has been used in small uncontrolled pilot studies). The findings of this study will provide a mechanistic rationale for instituting insulin in youth with CF and pre-diabetes, and will inform both research studies and clinical practice as to the best regimen for insulin delivery in this population.
- Both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.
- The researcher confirms that the minor subject ages 8-17 may choose not to participate and his/her dissent will be honored.
- The child ages 8-17 will sign an assent form which will be kept along with the parental consent. If the subject turns 18 before completion of the study, they will be reconsented and sign a main consent form to be kept in their file.
- When enrolling minors, if there is more than one custodial parent or guardian, both must provide consent whenever possible. Documentation should demonstrate the informed consent process for each and the consent forms should be signed by both (if applicable) prior to any study procedure. The entire process must be completed, including the return of the second parent's signed consent document for signature by the person obtaining consent, prior to initiating any study procedures.

Ideally, both custodial parents or guardians would be present in person at a study visit. However, if one parent is unable to attend a visit in person the signature can be obtained on a remote basis. In general, this is done by providing the study materials (handbook, consent forms) to the parent and then speaking to the second parent by phone to provide fully informed consent.

After discussing the study and answering questions, the signed consent form can be returned (sent via mail, email, or fax) or brought to the study visit by the other responsible parent. If the signature dates are different (which is likely), an explanation that the remote signature was collected off site should be noted in the source document. If, in rare cases, one of the custodial parents is not available despite efforts to reach them (e.g. active duty), this should be documented in a note to file.

19.3 Non-English Speaking Participants: We have a translated parental consent form for those patients that have Spanish speaking parents to abide by our unexpected encounter that we will have used the short form on.

## 20. Setting

*20.1.* Research Sites:

- Dr. Moran maintains the CF OGTT Database and follows these patients clinically and will identify potential subjects through this.
- Research procedures will take place at the University of Minnesota Health Clinical Research Unit (M Health CRU) as well as at the Delaware Clinical Research Unit (DCRU).
- School-aged participants who are randomized into the pre-meal insulin arm will require the school to assist in the proper storage and overseeing the administration of the required insulin dosage.

## 21. Multi-Site Research

*21.1.* Study-Wide Number of Participants: 100-- number is high in case of drop outs, especially due to acute illnesses which are common in CF patients

*21.2.* Study-Wide Recruitment Methods: N/A

*21.3.* Study-Wide Recruitment Materials: N/A

## 22. Resources Available

Resources Available: We have a team of 8 research coordinators to do research for this research. We have one full time dedicated coordinator, regulatory specialist, and lab coordinator to assist in carrying out this protocol. We have access to the cystic fibrosis registry and colleagues to help us recruit potential participants. All of the research visits will take place at the University of Minnesota research units where they have all of the necessary equipment and staff to carry out the visits.

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