Biomarkers and Tests of Pancreatic Function

~or~

“Perspectives on a Key Issue”

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CF Survival Age

Median Predicted Survival Age 1986-2015 In 5 Year Increments

Demographic Trends
Number of Children and Adults with CF, 1986-2015

The decrease in the number of individuals in 2003 is due to the a delay in obtaining informed consent forms before the close of the calendar year in some CF Care Centers.

Cystic Fibrosis Foundation Patient Registry 2015 Annual Data Report, Bethesda, Maryland ©2016 Cystic Fibrosis Foundation
Suboptimal Enzyme and Nutrition Use

- 20% of 75 children took enzymes after eating events most (8%) or some (12%) of the time
- 59% below energy intake goal (120% EER)
- 72% below fat intake goal (40% fat)

Pancreatic Disease

- Acute Pancreatitis
- Chronic Pancreatitis (CF, Hereditary, Idiopathic)
- Shwachman-Diamond Syndrome
- Pearson Marrow-Pancreatic Syndrome
- Johanson-Blizzard Syndrome
- Lipase, Colipase, Combined Deficiency
- Surgical Resection
- Celiac Disease, HIV/AIDS
Approach to Fat Malabsorption

- Chronic (> 14 days) diarrhea
- 3-day/72 hr stool fat content
  - Stool and diet record
  - Coefficient of fat malabsorption
    7% fat loss
- Not: Steatocrit, acid steatocrit, microscopic analysis (fat globules)
- Fecal elastase
- Serum immunoreactive trypsinogen
- Others: $^{13}$C, $^{14}$C-breath test, MBT, others
Key Issues

• How to diagnose exocrine pancreatic insufficiency (PI)?

• How to determine efficacy of treatment (or change over time) of PI?
What is Pancreatic Insufficiency?

• Loss of up to 90% of pancreatic function with no/little clinical impact
• Based on pancreatic lipase secretion
• Digestion, absorption, metabolism
• Resulting nutritional status (weight, height, BMI, fat, muscle)
• Fat absorption → calories
• Fat soluble vitamins (A,D,E,K)
• Essential fatty acids (linoleic acid, α-linolenic acid)
• Bile acid circulation
Coefficient of Fat Absorption (CFA) Experience in CF

- 72-hr stool collection
- Complete food intake record over same period for fat intake
- Fat extraction from stool for fat loss
  - Homogenization, aliquot, specialty lab (Mayo)
- CFA ≥ 93% in healthy people
- Typical CFA for CF and PI = 83-85% (group mean)
- Individual values for CFA range from 40-50% with no enzymes (lingual and gastric lipase) to 90+%
CF Failure to Optimize Fat Intake and Absorption

- Suboptimal weight, length/height, BMI
- Reduced fat stores and muscle mass
- Delayed onset of puberty
- Suboptimal essential fatty acid status with some deficiency
- Suboptimal fat soluble vitamin status in spite of CF-specific vitamin use
Need Better Methods to Determine if Clinical Interventions are Efficacious

• Increases in enzymes and fat intake
  - Weight gain (fat and muscle)
  - Linear growth
  - Pubertal progression
  - Essential fatty acid and fat soluble vitamin status

• Treat for bacterial overgrowth
• Add acid suppression medication
• No usefully routine chemistry
Malabsorption Blood Test (MBT)

- Free fatty acids vs triglycerides differ in rate of absorption
- Odd-chain fatty acids have low concentration in the fasting state and are easy to detect in plasma (gas liquid chromatography)
- Pentadecanoic acid (PA) n=15 carbon length, and a free fatty acid
- Triheptadecanoin acid (THA) three, n=17 carbon length heptadecanoic acids (HA) as a triglyceride (THA)
- PA is rapidly absorbed as a free fatty acid
- THA, a triglyceride, must be digested by lipase to yield HA, free fatty acid

MBT Protocol

- Early morning fasting setting
- No diary products for 24 hrs prior to MBT
- 12 hour overnight fast except water
- Blood samples at baseline then hourly – 1 to 8 hrs post-test meal
- Test meal – high fat breakfast drink with PA and THA
- Then, only water, non-caffeinated, non-caloric beverages until after 6th hr sample (≈ 2:00pm lunch)
- Standardized lunch (1000 kcal, low fat)

MBT Test Meal

• 550 kcal, 32g fat, 52% kcal from fat (high fat CF meal plan) in 8 oz warm drink

• Soy milk based Scandishake®, microlipids, HA and THA

• Test meal consumed with five minutes

PA and HA Concentrations

- Plasma samples x 9 over 8 hours
- Standard gas chromatographic methods
- Inter-assay variability (%CV) with low, medium and high concentration PA: 2.9%, 2.6 %, 3.1%,
  HA: 2.6%, 4.0%, 3.9%
- Moment-based pharmacokinetic analysis (WinNonLin)
- Variables included for PA and HA
  - baseline and max concentrations - $C_{\text{max}}$
  - area under the curve (0 to 8 hrs) - AUC

Four MBT Studies

- Lipase Inhibitor Study (on/off Orlistat-pancreatic lipase inhibitor medication) in healthy subjects\(^1\)
- CF Pharmacokinetics Study (on/off PERT) in clinically stable subjects with CF and PI\(^1\)
- Timing of enzymes and absorption (30 min pre-meal, at meal initiation, 30 min post-meal, 60 min post-meal) in clinically stable subjects with CF and PI\(^2\)
- Gating Mutation Study – CF subjects pre- and post-ivacaftor treatment (Stallings et al., 2017, in review)

Lipase Inhibitor Study

• MBT demonstrated a significant reduction in absorption of HA with pancreatic lipase inhibition (Orlistat) in healthy subjects.

• Both the amount of HA absorbed (based on AUC and Cmax) and the ratio of HA absorption to PA absorption were significantly reduced.
  - 65% reduction in HA AUC and 71% reduction in HA Cmax.

• PA absorption, the free fatty acid, was not affected by the pancreatic lipase inhibitor.

Lipase Inhibitor Study

*n=15 Healthy Subjects*

**PA → FFA**

**THA → HA (lipase)**

Stallings, et al. 2013
$C_{\text{max}}$ and Area under Curve: HA/PA Ratio

Source: Stallings, et al. 2013
CF Pharmacokinetics Study

• MBT demonstrated a significant reduction in absorption of HA with reduction in pancreatic lipase activity (off enzymes)

• Both the amount of HA absorbed (AUC and Cmax) and the ratio of HA absorption to PA absorption were significantly reduced
  - Virtually no absorption of HA without enzymes

• PA absorption, the free fatty acid, was not affected by use of pancreatic enzymes

CF and PI: On/Off Enzymes

\[ n=6: 2.5 \text{ PA} \]

\[ n=3: 5\text{g THA} \]

\[ n=3: 8\text{g THA} \]

Stallings, et al. 2013
CF and PI: On/Off Enzymes: HA/PA Ratio

C_{\text{max}}\) HA/PA Ratio

PUC

AUC HA/PA Ratio

Stallings, et al. 2013

The Children's Hospital of Philadelphia
Subjects with CF vs. Healthy Subjects

- PA absorption (free fatty acid) was similar for healthy subjects and subjects with CF
- Based on parameter estimates from the pharmacokinetics modeling, PA bioavailability was 1.07 (95%CI, 0.827, 1.42) for subjects with CF compared to healthy subjects, was not different
- HA absorption (triglyceride) was significantly less in subjects with CF taking pancreatic enzymes than in healthy subjects
- HA bioavailability was 0.606 (0.483, 0.823) for CF, or ~61%
- For subjects with CF not taking pancreatic enzymes, there was little to no absorption of HA

Pharmacokinetic Models: Timing of Enzymes Study

Mascarenhas, et al. 2015
Timing of Enzymes in CF

- Taking pancreatic enzymes at initiation of meal (MBT) was optimal for absorption of THA/HA in subjects with CF.
- HA absorption was slightly decreased by a factor of 0.911 (0.710, 1.12) when enzymes taken 30 minutes prior to meal.
- With enzymes taken 30 or 60 minutes after the meal, HA absorption was decreased by factors of 0.829 (0.664, 0.979) and 0.78 (0.491, 1.13) compared to when taken with the meal.
- Loss of about 80% of fat.

Gateway to Weight Gain Study

- Subject with CF and CFTR gating mutations with clinical indications for treatment with ivacaftor (n=24)
- USA, Canada and Italy based enrollment with all study visits at the CHOP CTRC
- Aim to determine the mechanisms of treatment-related weight gain
- Energy balance: Intake, REE, TEE
- Gut Absorption: CFA, MBT
- Gut inflammation: Fecal calprotectin
- Pancreatic function: Fecal Elastase
- Outcomes compared by pancreatic status groups:
  - Pancreatic insufficient (PI) vs. sufficient (PS)
Gateway to Weight Gain: PA and HA Concentration Time Profiles
Gateway to Weight Gain Study: MBT Results Before and After Ivacaftor treatment

- HA:PA AUC ratio before and after 3-month ivacaftor
- Total intent-to-treat sample (left, n=22)
- Stratified by pancreatic status (right, PI = 16, PS = 6)
- P values compare PS with PI within time point

Stallings, et al. 2017, in review
Gateway to Weight Gain Study

• Using intent-to-treat analysis, absorption curves for PA and HA for 22 subjects with CF and gating mutations were similar before and after ivacaftor.

• HA:PA AUC ratio did not increase significantly with ivacaftor in the group as a whole. The apparent increase for the six subjects with PS did not reach significance.

• The HA:PA AUC ratio was significantly higher at both time points in PS vs. PI subjects, and particularly after ivacaftor treatment (p=0.0001).
Summary

• Managing EPI diagnosis is very important in pediatric and adult care

• MBT has promise to support these needs:
  - CF (PI, PS, different mutations)
  - Chronic pancreatitis (study in progress)
  - Aging, frail patients
  - Short bowel syndrome (study in progress)

• Changes in the amount of dietary fat absorbed will be informative in research, and potentially in clinical care