Malabsorption: The laboratory's contribution to the unsettled gut

Chemistry Seminar
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The University of Utah School of Medicine adheres to ACCME Standards regarding industry support of continuing medical education.

Speakers are also expected to openly disclose intent to discuss any off-label experimental or investigational use of drugs, devices, or equipment in their presentations.

This speaker has nothing to disclose.
An unsettled stomach

- 9 month old girl
- Skin rash $\rightarrow$ antibiotics
- Diarrhea next day
- Switch to soy-based formula, then protein hydrolysate
- Hospitalized

Learning Objectives

By the end of the session participants should be able to:

- Recall the anatomic location and physiological processes of digestive organs
- List several causes of malabsorption
- Suggest appropriate laboratory tests to aid in the evaluation of suspected malabsorption
Outline

- Review of the digestive system
  - Gastrointestinal anatomy and physiology
  - Mechanisms of nutrient breakdown
  - Nutrient absorption
  - Causes of abnormal function

- Malabsorption
  - Symptoms
  - Laboratory evaluation
  - Management
Digestion and Absorption

- The process by which nutrients are consumed, broken down, absorbed and transported to other parts of the body

- Mechanical, chemical processes

- Three phases:
  - Luminal → breakdown, solubilization
  - Mucosal → movement of nutrients into GI cells
  - Transport → distribution of nutrients throughout the body

- Allows food nutrients to be utilized for energy and growth
Format of Nutrients
- Gastrointestinal system
- Long tube
- Lumen open to external environment
The Digestive System

- Deglutition
- Mastication
- Absorption
- Defecation

Physical processes
Chemical processes
Deglutition
Peristalsis
Absorption
Defecation

The Digestive System

Physical processes
Chemical processes
Deglutition

Peristalsis

Bile:
- Produced in liver
- Stored in gallbladder
- Fat emulsification

Saliva → moisture, amylase

Pancreatic secretions:
- Bicarbonate
- Proteases
- Lipases
- Nucleases

Breakdown & absorption
3 sections:
- Duodenum
- Jejunum
- Ileum

Produces:
- Mucus
- Enzymes
- Hormones

4 sections:
- Ascending
- Transverse
- Descending
- Sigmoid

Water
Sodium
Storage
Carbohydrate Digestion

- Digestion begins in mouth
  - Salivary Amylase

- Continues in small intestine
  - Pancreatic Amylase

- And at the brush border
  - Disaccharidases
    - Lactase
    - Sucrase-Isomaltase
    - Maltase-glucoamylase
    - Palatinase
Protein Digestion

- Pepsinogen is converted to pepsin in the stomach
  - Protein $\xrightarrow{\text{Pepsin}}$ Peptides

- Pancreatic proteases are activated in the small intestine
  - Proteins $\xrightarrow{\text{Trypsin, Chymotrypsin, Elastase, Carboxypeptidase}}$ Oligopeptides $\xrightarrow{\text{Peptidase}}$ Dipeptides $\xrightarrow{\text{Peptidase}}$ Amino acids

- Small intestine also secretes peptidases
Fat Digestion

Mixing & Bile salts → micelles → Lipase → Fatty acids, Monoglyceride, Glycerol
Nutrient Absorption
Nutrient Absorption

- **To Liver**
  - GLUT2
  - SGLT1
  - GLUT5
  - GLUT2

- **To thoracic duct**
  - Amino acids
  - AA transporter
  - PEPT1
  - Dipeptides
  - H^+ / H_2O

- **Short-chain fatty acids**
- **Longer fatty acids**
- **Cholesterol**

Based on: Kapit et al. 2000, p. 79
Causes of Malabsorption

Digestion
  *Luminal*

Absorption
  *Mucosal*
Causes of Malabsorption

**Impaired Digestion**

- Abnormal physical processes
  - Inadequate chewing
- Inadequate digestive secretions
  - Bile acid disorders
  - Obstructions (bile or pancreatic duct)
  - Liver dysfunction
  - Bacterial overgrowth

- Enzyme insufficiency
  - Pancreatic insufficiency
    - Chronic pancreatitis
    - Cystic Fibrosis
    - Shwachman-Diamond Syndrome
  - Zollinger-Ellison Syndrome
  - Disaccharidase deficiency
  - Congenital/genetic deficiencies
Causes of Malabsorption

Impaired Absorption

- Physical damage
  - Short bowel syndrome
  - Bowel obstructions
  - Intestinal tissue damage
    - Celiac disease
    - Crohn's disease
  - Whipple's disease

- Transporter/cofactor deficiencies
  - Hartnup's → amino acid transport
  - SGLT-1 → glucose/galactose transport
  - Intrinsic factor → Vitamin B12 transport
Symptoms of Malabsorption

- Undigested/unabsorbed molecules in the GI tract
  - Undigested/unabsorbed molecules in the GI tract:
    - Nutrients reach colon
      - Excreted in feces
      - Steatorrhea
      - Protein
      - Sugars
  - Bacterial fermentation
    - Flatulence
    - Acid production
Symptoms of Malabsorption

- Undigested/unabsorbed active molecules in the GI tract
  - Diarrhea
  - Flatulence
  - Bloating

- Malnutrition, failure to thrive
  - Nutrient insufficiency
  - Weight loss
  - Wasting
  - Fatigue
  - Anemia

Symptoms are non-specific
An unsettled stomach

- 9 month old girl
- Skin rash → antibiotics
- Diarrhea next day
- Switch to soy-based formula, then protein hydrolysate
- Hospitalized
  - Staphylococcal scalded skin syndrome
  - Negative: toxins, ova, parasites, bacteria
  - Normal: CBC, electrolytes

Laboratory Evaluation of Malabsorption

- Look for unabsorbed nutrients in feces and urine
  - Sugars, fat, protein
- Look for absence of nutrients in circulation
  - Oral glucose load
- Look for indirect evidence of malabsorption
  - Stool pH
  - Hydrogen breath test
  - Fat-soluble vitamin deficiencies
- Look for the presence and activity of digestive enzymes
  - Disaccharidase activity
  - Fecal elastase, trypsin
  - Enzyme levels in duodenal aspirates
- Look for evidence of GI damage
  - Xylose absorption test
  - Endomesial and gliadin antibodies
  - Inflammatory markers
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  - Stool analysis: 0.75-2% reducing substances
  - pH 5
- Diarrhea remitted when oral feedings were stopped; resumed when oral feedings were resumed

Laboratory Evaluation of Malabsorption

- Look for unabsorbed nutrients in feces and urine
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D-xylose absorption
Mucosal permeability of small intestine

- Pentose monosaccharide passively absorbed in proximal small bowel
- Excreted in urine

Procedure:
Overnight fast
5 or 25 g oral dose of D-xylose
5 hour urine collection
(1 or 2 hour blood collection)
oral xylose dose

serum → serum → serum → urine

5 hrs

0.3 mM xylose

1.7 mM glucose

Eberts et al. 1979
Reference Intervals (adults, 25 g dose)

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<table>
<thead>
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<tbody>
<tr>
<td>2 hr serum</td>
<td>32-58 mg/dL</td>
</tr>
<tr>
<td>5 hr urine – fraction</td>
<td>14-40%</td>
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<tr>
<td>– amount</td>
<td>3.5-10 g/hour</td>
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Craig & Ehrenpreis, 1999
Laboratory Evaluation of Malabsorption

- Look for unabsorbed nutrients in feces and urine
- Look for absence of nutrients in circulation
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- Look for the presence and activity of digestive enzymes
- Look for evidence of GI damage
Fecal Fat Testing
Evidence of unabsorbed nutrients

- Steatorrhea → pancreas, bile acid, damage, transport, mixing diagnosing fat malabsorption

- "Gold standard" for diagnosis

- Treatment monitoring

- Method history: solvent extractions, titrimetric or gravimetric, FTIR

Procedure:
3 day stool collection
Normal (50-150 g/day) fat diet
No barium, charcoal or non-digestible fat intake
Sample is weighed and dried

Method:
NMR
Calibrated
Quantitation of % fat
Calculated weight/day result
**H¹ NMR**

- Nuclear magnetic resonance

- Proton in a magnetic field excited by radio-frequency pulse resonates at a particular frequency which then decays over a period of time

- Signal decay is slower when protons are in lipids than in other substances
  - Signal isolation
  - % Fat determined using calibration curve

| Quantitative Fecal Fat Reference Interval (72 hr collection) |
|-------------------|----------------|
| 0-5 years         | 0 - 2.0 g/24h   |
| ≥ 6 years         | 0 - 6.0 g/24h   |
Laboratory Evaluation of Malabsorption

- Look for unabsorbed nutrients in feces and urine
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**Fecal Elastase**

Pancreatic exocrine function, protease enzymes

- Produced by pancreas
  
  \[
  \text{proelastase} \xrightarrow{\text{trypsin}} \text{elastase}
  \]
  
  *duodenum*

- Serine protease, hydrolyzes amide and ester bonds

- Remains intact and active in the intestine

- Concentrated in feces versus duodenal fluid

Fecal Elastase

- Enzyme-linked immunoassay
- Stool homogenates
- Double-sandwich, signal amplification
- Species and tissue-specific antibodies

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<th>Pancreatic Elastase (μg/g feces)</th>
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<tr>
<td>Normal</td>
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<tr>
<td>Mild-moderate insufficiency</td>
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<tr>
<td>Severe insufficiency</td>
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Disaccharidase Assay

Brush-border disaccharidase activity

• Lactase
  \[ \text{lactose} \rightarrow \text{glucose} + \text{galactose} \]

• Sucrase-Isomaltase
  \[ \text{sucrose} \rightarrow \text{glucose} + \text{fructose} \]
  \[ \text{palatinose}^* \rightarrow \text{glucose} + \text{fructose} \]
  \[ \text{maltose} \rightarrow \text{glucose} + \text{fructose} \]
  \[ \text{isomaltose} \rightarrow \text{glucose} + \text{fructose} \]

• Maltase-Glucoamylase
  \[ \text{maltose} \rightarrow \text{glucose} + \text{fructose} \]
  \[ \text{amylose} \rightarrow \text{glucose} \]

• Trehalase
  \[ \text{trehalose} \rightarrow \text{glucose} + \text{fructose} \]

* a.k.a. isomaltulose
**Dissacharidase Assay**

- **Sample:** ~ 5 mg intestinal biopsy
- **Procedure:** Homogenize tissue

**Test individual dissach activity**

- **Lactose**  
  lactase → glucose + galactose

- **Sucrose**  
  S-I → glucose + fructose

- **Palatinose**  
  S-I → glucose + fructose

- **Maltose**  
  S-I / M-G → glucose + glucose

- **Saline**  
  → blank/endogenous sugar

**Cobas Glucose Assay**

- **Glucose** + **ATP**  
  hexokinase → G-6-P + ADP

- **G-6-P** + **NADP⁺**  
  G-6-PD → gluconate-6-P + NADPH + H⁺

  
  **Assay total protein**
  
  *Lowry-based method*
For each sample:

- Total protein
- Glucose derived from lactose (Lactase, β-1,4-activity)
- Glucose derived from sucrose (S-I, α-1,2-activity)
- Glucose derived from palatinose (S-I, α-1,6-activity)
- Glucose derived from maltose (S-I, α-1,4-activity)
- Baseline glucose in sample (blank)

Enzyme activity (rate of glucose formation) normalized to total protein

\[ \mu \text{mol/min/g total protein} \]
Primary disaccharidase deficiency can be established only in the absence of intestinal injury

- **Lactase deficiency**
  - Age-dependent onset
  - 80-100% prevalence in some groups

- **Sucrase-isomaltase deficiency**
  - Congenital, gene mutations affect processing
  - Rare, 0.2-5% prevalence

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- Diarrhea remitted when oral feedings were stopped; resumed when oral feedings were resumed

- Disaccharidase testing showed low sucrase-isomaltase activity

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<th>Disaccharidase Activity (μmol/min/g)</th>
<th>Reference Interval</th>
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<tr>
<td>Lactase 33.4</td>
<td>≥ 15</td>
</tr>
<tr>
<td>Maltase 20.3</td>
<td>≥ 100</td>
</tr>
<tr>
<td>Sucrase 0.6</td>
<td>≥ 25</td>
</tr>
<tr>
<td>Palatinase 3.2</td>
<td>≥ 5</td>
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Management of Malabsorption

- Treat underlying conditions
  - Antibiotics, surgery, anti-inflammatory agents

- Nutritional supplementation
  - Vitamins and minerals
  - Parenteral nutrition

- Avoid sources of offending substances
  - Disaccharide-containing foods (milk, table sugar)
  - Gluten-free diet

- Enzyme Replacement
  - Disaccharidases
  - Pancreatic enzymes

- Pre-treated/modified foods
  - Yogurt & aged cheese (bacterial fermentation)
  - Lactose-reduced milk
  - Shorter-chain fats → less dependent on bile and lymphatics
An unsettled stomach

- 9 month old girl
- Skin rash → antibiotics
- Diarrhea next day*
- Switch to soy-based formula, then protein hydrolysate**
- Cow's milk formula did not produce symptoms
- Avoiding sucrose or isomaltose-containing formula prevented symptoms

* The antibiotic preparation contained sucrose
** Alternative formula preparations contained corn syrup solids
Conclusions

- Causes of malabsorption may arise from disruption of physical and/or chemical processes of digestion as well as impairments in nutrient absorption.

- Laboratory methods employing a wide variety of methodologies can help in the evaluation of suspected malabsorption and help to identify the underlying causes.

- Patient management will depend on the underlying cause and can include dietary modification as well as supplementation.

Thanks! Any questions?

Special thanks to the Sp Chem and PAFT labs.
References


The Merck Manual, online: www.merckmanuals.com

ARUP Consult

MasterControl