Nutritional Assessment – Vitamin Testing

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Vitamins are essential nutrients required for human health. In the body, vitamins function as enzymatic cofactors and antioxidants, and are active in metabolism and energy production. Vitamins are not synthesized endogenously and must be ingested regularly to maintain health and prevent deleterious consequences of deficiency. Laboratory testing is performed to assess nutritional status and to monitor therapeutic supplementation of vitamins.
Objectives

• Define nutrition and state the role of the clinical laboratory in nutritional assessment.

• Describe appropriate use and interpretation of laboratory testing for vitamins.

• Review requirements for collection, processing, and transport of specimens for nutritional assessment.

• Summarize analytical methods for vitamin assessment.

• Discuss vitamin B$_1$ deficiency in an unexpected clinical setting.
Outline

- Overview of nutrition and nutritional assessment
- Use and interpretation of laboratory testing for vitamins
- Requirements for specimen collection, processing, and transport
- Analytical methods for vitamin measurement
- Clinical case study – Vitamin deficiency
The Big Picture

NUTRITION

- The utilization of nutrients to support and maintain health

NUTRIENTS

- Substances required for essential metabolic processes

- Food intake, absorption, assimilation, biosynthesis, catabolism, excretion
Nutritional Assessment

History – Dietary, medical, social
  – Information on intake and requirements

Physical examination
  • Anthropometric measurements
    – Height, weight, triceps skinfold thickness, mid-arm muscle circumference
    – Estimation of protein and fat stores, growth
  • Body composition analysis

Biochemical tests
  – Laboratory evaluation of nutrients in body fluid specimens
Nutrient Categories

Macronutrients
- Intake >1 gram/day
  - Proteins
  - Carbohydrates
  - Fats
  - Energy
  - Structural and functional components

Micronutrients
- Intake <1 milligram/day
  - Vitamins
  - Trace elements
  - Ultra-trace elements
  - Metabolic cofactors and coenzymes
  - Antioxidants
Vitamins - Definition

- Organic compounds
- Small amounts (<1 mg/day) are required for essential physiological processes
- Not synthesized in the human body
- Absence/deficiency produces disease symptoms that are corrected by restoring the nutrient to adequate concentration
Vitamins - Characteristics

• Chemically unrelated substances
• Different physiological functions
• Obtained from different food sources

• Vitamins may be single compounds
  – Vitamin C (Ascorbic acid)

• Some vitamins are families of related compounds
  – Vitamin A (Retinol, retinal, retinaldehyde)
  – Vitamin E (Tocopherols, tocotrienols)
Vitamins - Classification

• SOLUBILITY
  – Water or lipid soluble

• Solubility affects
  – Absorption and transport
  – Storage, toxicity, excretion
  – Response to particular disease or injury conditions
Vitamins

WATER SOLUBLE

Vitamin $B_1$ - Thiamine, thiamine phosphates
Vitamin $B_2$ - Riboflavin, other flavins
Vitamin $B_3$ - Niacin, nicotinic acid, nicotinamide
Vitamin $B_6$ - Pyridoxine, pyridoxal, pyridoxamine
Vitamin $B_{12}$ - Cyanocobalamin
Vitamin C - Ascorbic acid, dehydroascorbic acid
Vitamins

LIPIID SOLUBLE

Vitamin A - Retinoids
Vitamin E - Tocopherols, tocotrienols
Vitamin K - Phylloquinones, menaquinones
Laboratory Testing
- Use and interpretation
Laboratory Testing

• Vitamin testing is appropriately used to assess nutritional status
  – Deficiency, sufficiency, or toxicity of particular individual vitamins or vitamers.

• Specific stipulations apply for
  – Specimens
  – Reference values
  – Use of test results
Use of Test Results

Nutritionists and dieticians usually think in terms of intake.

- What are the required amounts of the vitamins that an individual should consume as food or supplement to support health and prevent or recover from disease or injury?

Measures

- Dietary Reference Intake (DRI) – Reference values or estimates of dietary amounts of each essential nutrient.
- Recommended Dietary Allowance (RDA) – Average daily dietary amount sufficient for the nutrient requirements of most (97-98%) healthy people categorized by age, gender, and physiological need.

In the clinical lab, vitamin concentrations are measured in body fluids.

The relationship between intake and concentration may not be well-characterized.
Assessment of Status

Laboratory measurement of vitamin concentration can aid clinical assessment of patients

If

– Relationship to requirements for intake
– Relevant reference values

are known.
Reference Values

• Specimen
  – Type
  – Collection, processing, storage

• Subject condition
  • Fasting, non-fasting
  • Vitamin supplement use
  • Age, gender
  • Health, disease
  • Geography/culture - Diet
Assessment of Status

- **Specimens**
  - Whole blood, serum or plasma, urine

- **Measurement of extracellular concentration**
  - Micronutrients, including vitamins, perform biochemical functions within cells
  - Indirect and relatively insensitive indicator of nutrient status
  - Sufficient to determine deficiency or toxicity
  - In some cases, reliable index of status
Assessment of Status

• Vitamins = Photosensitive, labile compounds
  – Collection, transport, and storage conditions are critical for specimen integrity

• Vitamin concentrations in biological fluids
  – Picomolar (pmol/L) to micromolar (μmol/L)

• Analytical methods
  – Must be sensitive and specific for accurate results
Specimens

- Collection
- Processing
- Transport
Specimens

• Labile compounds
  – Sensitive to light and temperature

• Collection, transport, and storage critical

Specimen Preparation: Protect from light during collection, storage and shipment. Separate plasma from cells within 1 hour of collection.

Storage/Transport Temperature: Frozen. Separate specimens must be submitted when multiple tests are ordered.

Stability: After separation from cells: Ambient: Unacceptable; Refrigerated: Hours to days; Frozen (-20°C): Weeks, Frozen (-70°C): Several months
Specimen Requirements

Vitamin C
Specimen Preparation: Protect from light, centrifuge, transfer plasma and freeze within 1 hour of collection.

Storage/Transport Temperature: CRITICAL FROZEN AND LIGHT PROTECTED. Separate specimens must be submitted when multiple tests are ordered.

Vitamin A
Patient Preparation: Patient should fast for 12 hours and abstain from alcohol consumption for 24 hours prior to collection.

Vitamin B₆
Patient Preparation: Collect specimen after an overnight fast.
Vitamin assessment
- Laboratory methods
Laboratory Measurement

- **Mass assay**
  - How much of the vitamin is present?

- **Functional assay**
  - Does the vitamin work?

- **Excretion testing**
  - Is there excess vitamin?

- **Indirect assay**
  - Can the effect or lack of effect be observed?
Analytical Challenges

- Labile analytes
  - Sensitive to light and temperature
  - Collection, transport, storage critical

- Small amounts present
  - Picomolar/nanomolar concentrations
  - Large specimen volume required

- Large amounts present in some specimens
  - Disease-affected individuals or those on supplements
  - Broad Analytical Measurement Range required
  - Specimens must be diluted/repeated
    - Adds complexity and extends TAT
Methods

- Quantitative assays
  - Measurement of individual vitamins/vitamers
- Sample preparation
  - Removal of proteins
  - Other – Extraction, derivatization
- Chromatographic separation
  - Liquid chromatography (HPLC)
- Detection
  - UV/Visible spectrometry
  - Fluorometry
  - Electrochemistry
  - Mass spectrometry
Examples
Vitamin A
Vitamin E

alpha-Tocopherol
"Vitamin E"

gamma-Tocopherol

delta-Tocopherol

alpha-Tocotrienol

gamma-Tocotrienol

delta-Tocotrienol

beta-Tocopherol

beta-Tocotrienol
Vitamins A and E

Chromatographic results for vitamins A (retinol and retinyl palmitate) and E (α-tocopherol and γ-tocopherol) analyzed using the modified high throughput HPLC method.

Retention time and concentration of analytes: retinol (0.5 min, 1.15 μmol/L), retinyl palmitate (1.8 min, 0.04 μmol/L), α-tocopherol (1.3 min, 13.4 μmol/L), γ-tocopherol (1.2 min, 2.9 μmol/L), and retinyl acetate (0.8 min, 1.5 μmol/L) used as an internal standard (IS).
Vitamin K

**Koagulationsvitamin**
- Vitamin $K_1$ – Phylloquinones
  - Obtained from diet
- Vitamin $K_2$ – Menaquinone
  - Synthesized by gut microflora

**Physiological role**
- Required for carboxylation of glutamic acid residues
  - Coagulation factors II, VII, IX, X
  - Anticoagulant Proteins C and S
  - Other proteins (matrix gla-protein, osteocalcin)
Vitamin K

1 mL serum/plasma + internal standard

- Protein precipitation
- Liquid-liquid extraction
- Liquid-liquid extraction
- Solid-phase extraction
- HPLC

Method based on MacCrehan et al. 1988, 1995
Vitamin B₁ (Thiamine)
- Unexpected deficiency
# Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>- Retinoids</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>- Thiamine, thiamine phosphates</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>- Riboflavin, other flavins</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>- Pyridoxine, pyridoxal, pyridoxamine</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>- Ascorbic acid, dehydroascorbic acid</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>- Tocopherols, tocotrienols</td>
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<tr>
<td>Vitamin K</td>
<td>- Phylloquinones, menaquinones</td>
</tr>
</tbody>
</table>
Vitamin B₁

• Water soluble vitamin of the B group

• Requirements
  • 1.1 – 1.2 mg/d  Adult female/male

• Nutritional sources
  • Whole grains, wheat germ
  • Meats, fish, legumes, nuts
  • Fortified foods (grains, cereals)

• Homeostasis
  • Intestinal absorption – Active and passive
  • Transport (buffer) – Erythrocytes
  • Phosphorylation – Liver
  • Excretion – Urine
**Vitamin B₁**

- Essential co-factor for cellular energy metabolism
  - Bioactive form – Thiamine diphosphate (TDP)
  - Short half-life; limited tissue storage

- Consequences of deficiency
  - Impaired neurological and cardiovascular function
    - Dry Beriberi: Neurologic effects
    - Wet Beriberi: Heart failure
    - Wernicke-Korsakoff syndrome: Delirium, mental confusion

- Inadequate nutrition – At risk
  - Impoverished, elderly
  - Limited diet or impaired absorption
  - Chronic alcoholics
Vitamin B₁

- Vitamers
  - Thiamine (unphosphorylated)
  - Phosphate esters
    - Thiamine monophosphate (TMP)
    - Thiamine diphosphate (TDP)
    - Thiamine triphosphate (TTP)
<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Thiamine (nmol/L)</th>
<th>TMP (nmol/L)</th>
<th>TDP (nmol/L)</th>
<th>Total (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma (n=118)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.0</td>
<td>8.8</td>
<td>Not detected</td>
<td>16.9</td>
</tr>
<tr>
<td>Range</td>
<td>3.2 – 22.0</td>
<td>3.3 – 16.4</td>
<td></td>
<td>6.9 – 32.3</td>
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<tr>
<td><strong>Whole blood (n = 110)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.5</td>
<td>4.3</td>
<td>111.6</td>
<td>124.8</td>
</tr>
<tr>
<td>Range</td>
<td>3.3 – 12.2</td>
<td>1.6 – 8.3</td>
<td>70.3 – 178.6</td>
<td>75.2 – 193.8</td>
</tr>
</tbody>
</table>
# Observed concentrations - ARUP

## Thiamine vitamer concentrations

<table>
<thead>
<tr>
<th></th>
<th>DEFICIENT</th>
<th>SUFFICIENT</th>
<th>INCREASED</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole blood</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[TDP]</td>
<td>&lt;70 nmol/L 12%</td>
<td>70 – 180 nmol/L</td>
<td>&gt;180 nmol/L 7%</td>
</tr>
<tr>
<td>n = 105,158</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[T] + [TMP]</td>
<td>&lt;8 nmol/L 20%</td>
<td>8 – 30 nmol/L 64%</td>
<td>&gt;30 nmol/L 16%</td>
</tr>
<tr>
<td>n = 42,630</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reference intervals (RI) established using fasting specimens collected from self-reported healthy adults.

Davis 2015
Thiamine Deficiency

- Predisposing factors
  - Alcohol misuse and malnutrition
  - Cancer and chemotherapeutic treatments
  - **Gastrointestinal surgery**
  - Magnesium depletion
  - Recurrent vomiting, chronic diarrhea
  - Staple diet of polished rice
  - Systemic diseases
  - Unbalanced nutrition
  - Use of chemical compounds and drugs

Overweight BMI ≥25 kg/m²
Obesity BMI ≥30 kg/m²
Extreme obesity BMI ≥40 kg/m²
Obesity and Nutrition

• Obesity as a disease of **over-nutrition**
  • Excess high-calorie, low nutrient-dense processed foods high in fats and simple sugars
    • Energy dense foods - contribute to weight gain
    • Essential nutrients, vitamins, minerals missing

• Obesity as a disease of **malnutrition**
  • NHANES III survey
    • Multiple nutrient deficiencies more common in persons with obese BMI
Obesity Treatment

• Bariatric surgery
  • Roux-en-Y gastric bypass
    • Restrictive-malabsorptive procedure
    • Decreases size of stomach
    • Alters GI tract to bypass duodenum and jejunum

• Post-surgical consequences
  • Decreased oral intake
  • Decreased nutrient absorption
  • Possible nausea and vomiting

Shankar Nutrition 2010
Thiamine Deficiency

- Potential postoperative complication
- Patients may be thiamine deficient before surgery
- Thiamine requirements increased after surgery
- Early symptoms are common to many disorders
  - Fatigue
  - Irritability
  - Poor memory
  - Abdominal discomfort
  - Anorexia
  - Sleep disturbances
- Moderate deficiency may be not be diagnosed
Thiamine Deficiency

- Wernicke’s encephalopathy
  - Acute, neuropsychiatric syndrome
  - Characteristics
    - Mental status changes
    - Ocular abnormalities
    - Unsteadiness standing and moving
  - Possible poor outcome; potentially fatal
  - Usually develops 4 – 12 weeks postop
    - Range 2 weeks to 20 years
  - Treatment – Thiamine administration

- Korsakoff’s syndrome
  - Severe memory defects
  - Treatment – Little response to thiamine
Cases

- **Case 1**
  - **36F**
  - 4/28 Vitamin B₃ P 7 (8-30 nmol/L)
  - 7/31 Vitamin B₃ P <2
  - 9/09 Vitamin B₃ P 120
  - 9/12 Vitamin B₁ WB 242 (70-180 nmol/L)

- **Case 2**
  - **21F**
  - 3/05 Vitamin B₁ WB 35 (70-180 nmol/L)
  - 3/11 Roux-en-Y gastric bypass surgery
  - 3/23 Difficulty ingesting food, fluids
  - 5/10 Vitamin B₁ WB 15
  - 5/25 Vitamin B₁ WB 53
Guidelines

• Recommendations vary
  • Pre-operative screen for thiamine deficiency
  • Daily multivitamin and mineral supplements
  • Post-operative supplementation

• Testing
  • “Regular monitoring of serum nutrient levels starting 3 months post-surgery”
  • “Testing thiamine levels not necessary”

• Clinical laboratory guidance
  • Measurement of TDP in whole blood specimen