Nutritional Assessment – Vitamin Testing

Elizabeth Frank, PhD, D(ABCC)

Medical Director
Analytic Biochemistry, Calculi and Manual Chemistry, Mass Spectrometry
ARUP Laboratories, Inc.

Professor of Pathology
University of Utah Department of Pathology

October 24, 2016

Brief Description

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

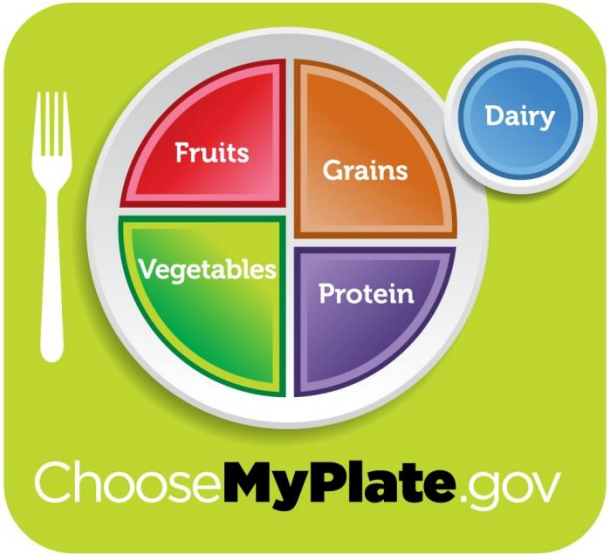
- The utilization of nutrients to support and maintain health

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

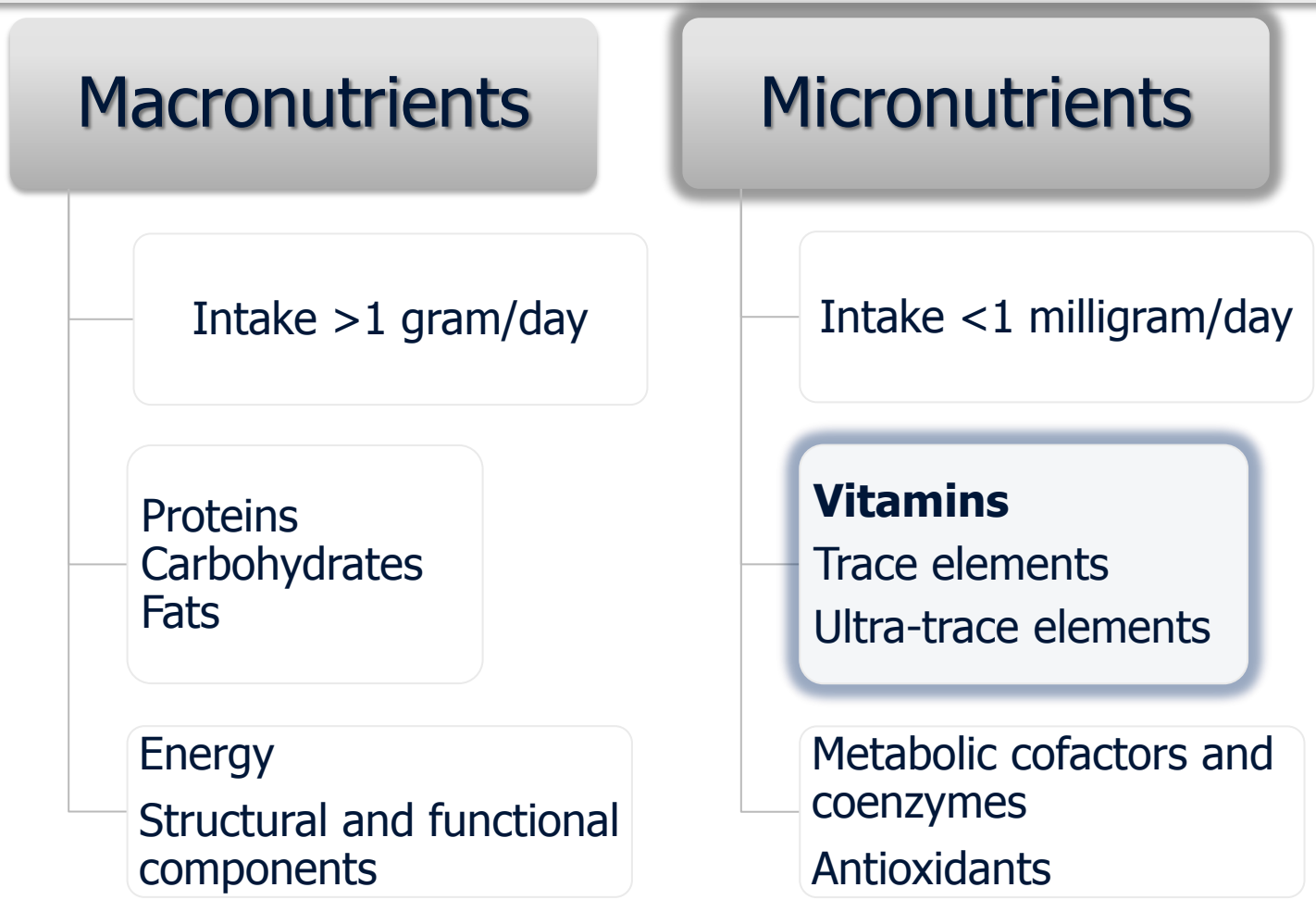
Physical examination

- 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



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

LIPID 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 B_6

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

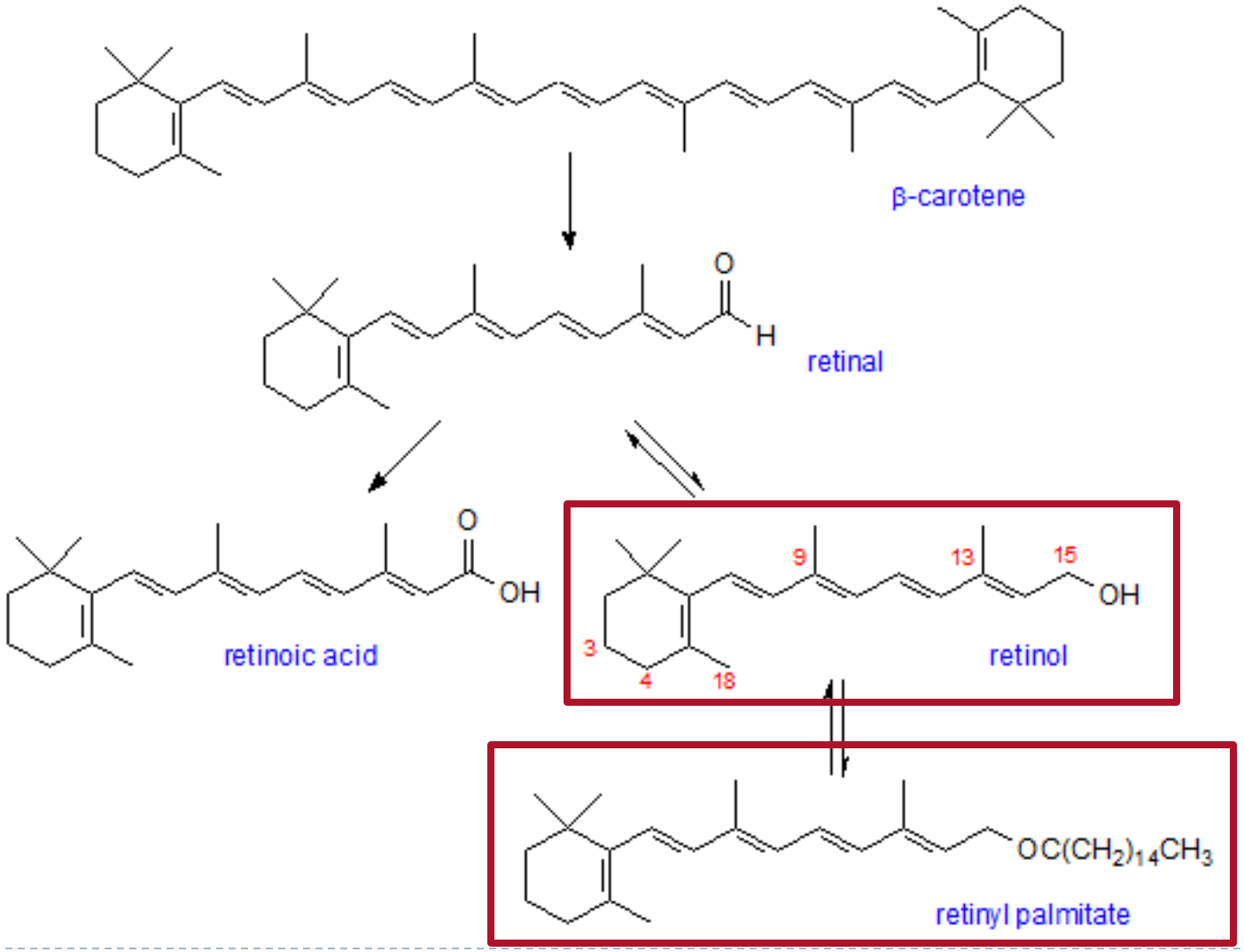
- Labile analytes
  - Sensitive to light and temperature
  - Collection, transport, storage critical
- Small amounts present
  - Picomolar/nanomolar concentrations
  - Large specimen volume required
- Large amounts present in some specimens
  - Disease-affected individuals or those on supplements
  - Broad Analytical Measurement Range required
  - Specimens must be diluted/repeated
    - Adds complexity and extends TAT

Methods

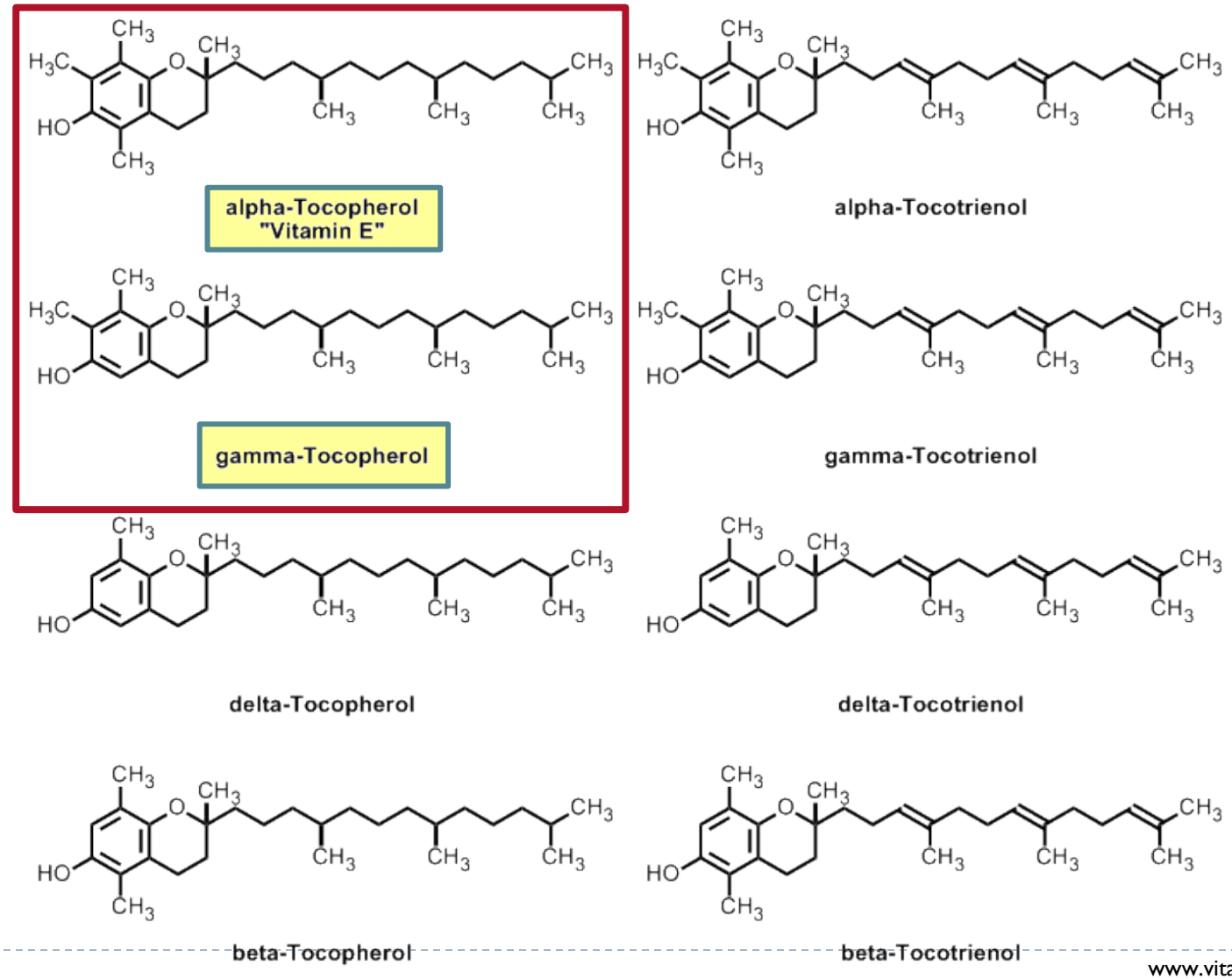
- Quantitative assays
  - Measurement of individual vitamins/vitamers
- Sample preparation
  - Removal of proteins
  - Other – Extraction, derivatization
- Chromatographic separation
  - Liquid chromatography (HPLC)
- Detection
  - UV/Visible spectrometry
  - Fluorometry
  - Electrochemistry
  - Mass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

Examples

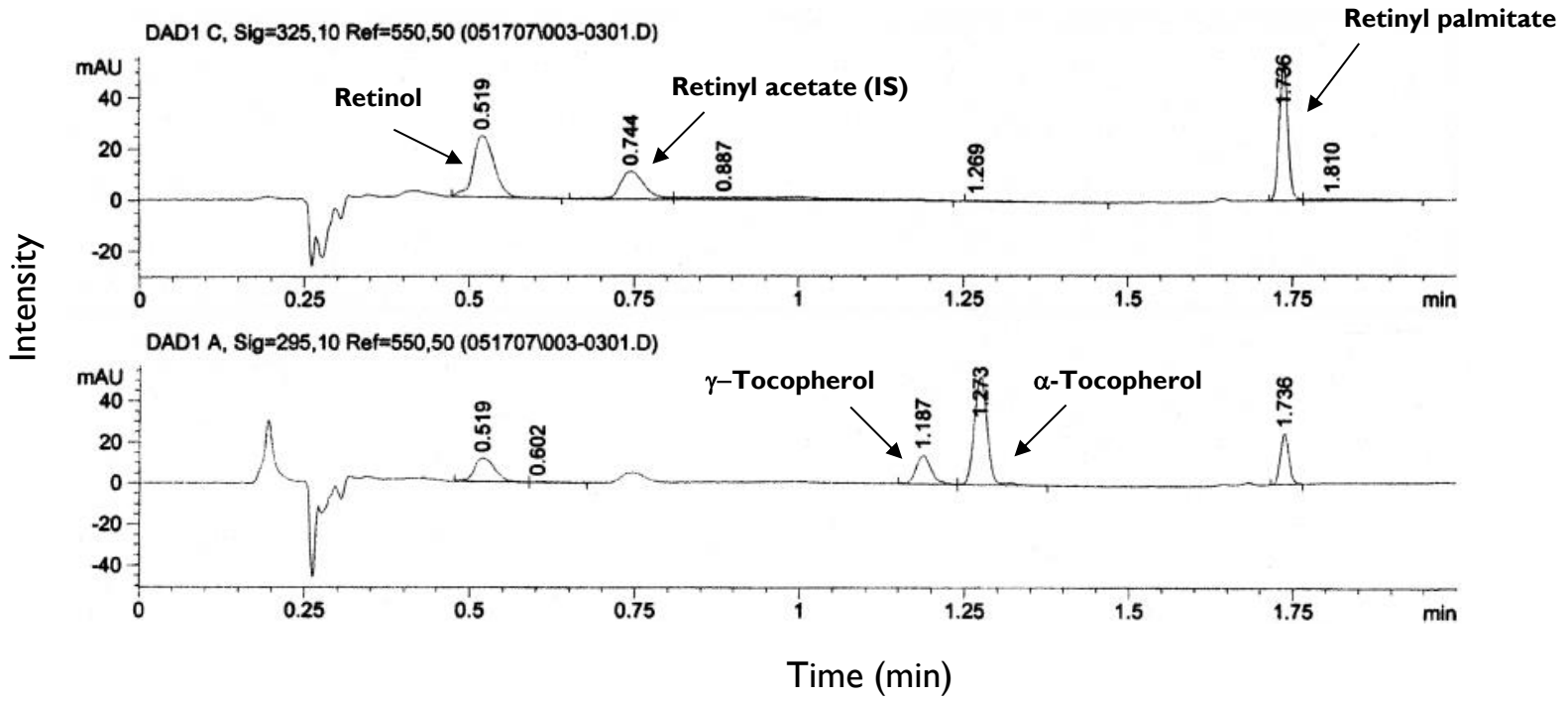
Vitamin A


β-carotene
O
H
retinal
O
OH
retinoic acid
9
13
15
OH
3
4
18
retinol
OC(CH2)14CH3
retinyl palmitate

Vitamin E


alpha-Tocopherol
"Vitamin E"
gamma-Tocopherol
alpha-Tocotrienol
gamma-Tocotrienol
delta-Tocopherol
delta-Tocotrienol
beta-Tocopherol
beta-Tocotrienol

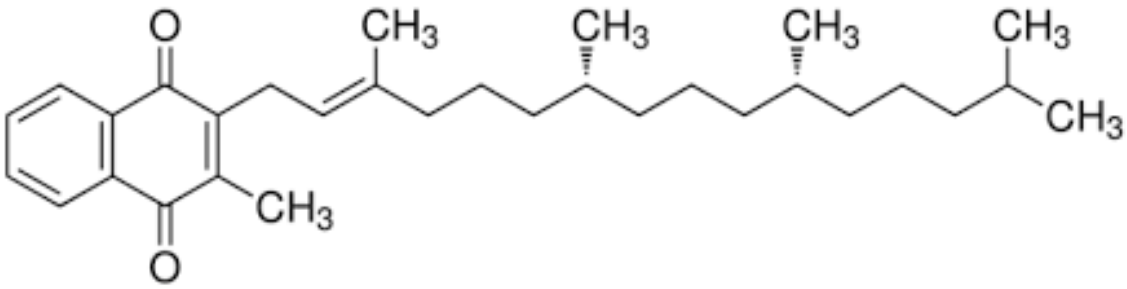
Vitamins A and E



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 (1.3min,13.4 μmol/lL, γ-tocopherol (1.2min, 2.9 μmol/L), and retinyl acetate (0.8 min,1.5 μmol/L) used as an internal standard (IS).

Johnson-Davis 2009

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

⇩

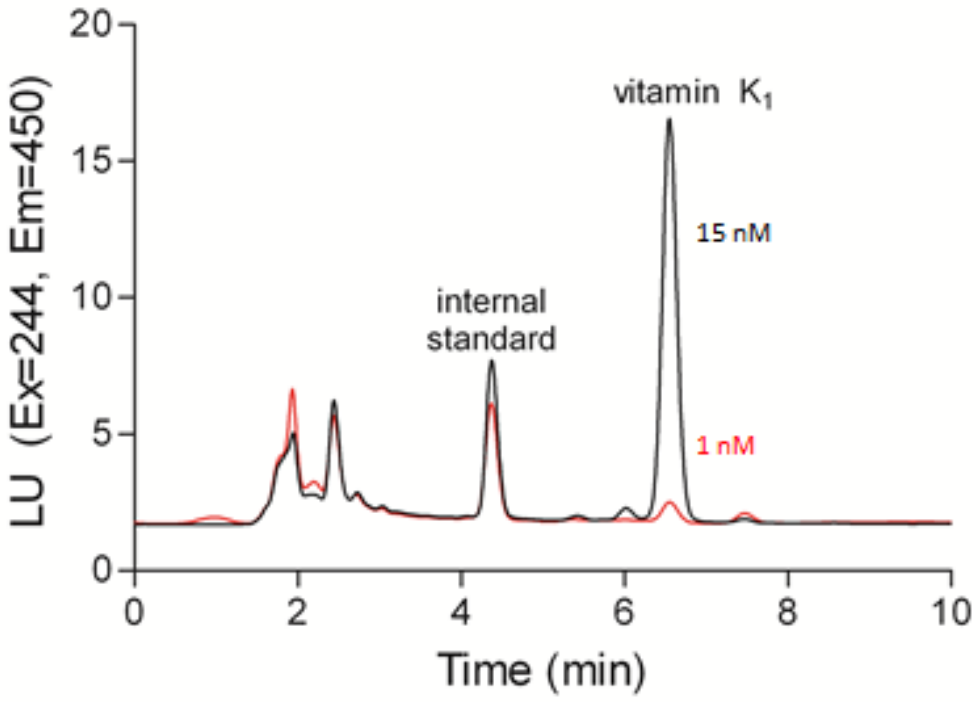
Liquid-liquid extraction

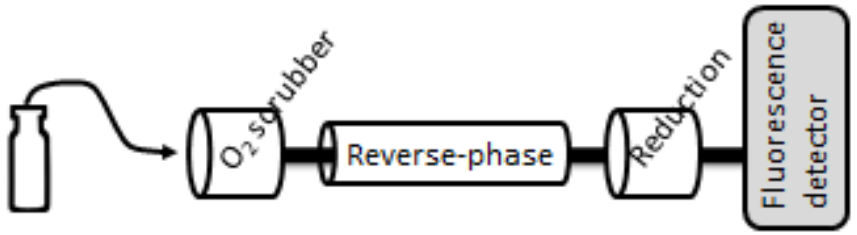
⇩

Solid-phase extraction

⇩

HPLC





Method based on MacCrehan et al. 1988, 1995

Vitamin B_1 (Thiamine)
- Unexpected deficiency

Vitamins

Vitamin A	- Retinoids
Vitamin B_1	- Thiamine, thiamine phosphates
Vitamin B_2	- Riboflavin, other flavins
Vitamin B_6	- Pyridoxine, pyridoxal, pyridoxamine
Vitamin C	- Ascorbic acid, dehydroascorbic acid
Vitamin E	- Tocopherols, tocotrienols
Vitamin K	- Phylloquinones, menaquinones

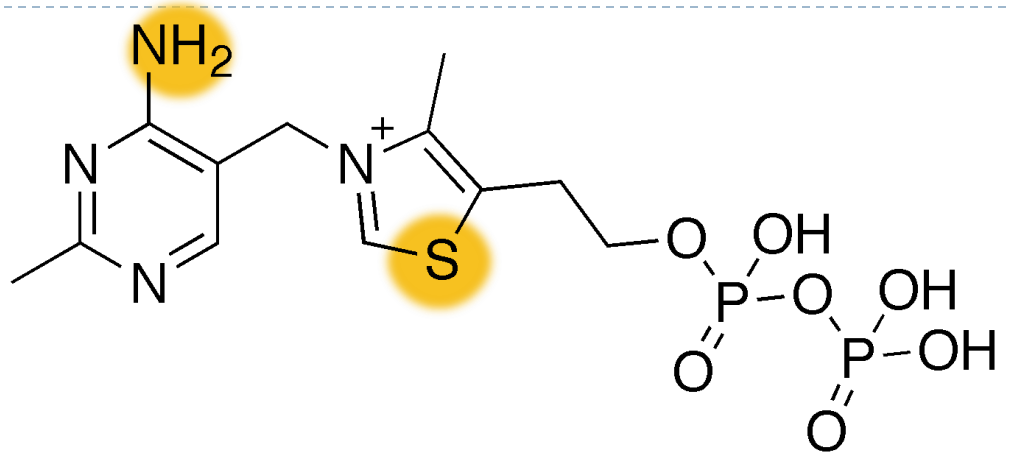
Vitamin B_1

- 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 B_1

- 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 B_1



- Vitamers
  - Thiamine (unphosphorylated)
  - Phosphate esters
    - Thiamine monophosphate (TMP)
    - Thiamine diphosphate (TDP)
    - Thiamine triphosphate (TTP)

commons.wikimedia.org

Vitamin B_1 vitamers by specimen type

	Thiamine (nmol/L)		TMP (nmol/L)		TDP (nmol/L)		Total (nmol/L)
Plasma (n=118)							
Mean	8.0	47.4%	8.8	52.6%	Not detected		16.9
Range	3.2 – 22.0		3.3 – 16.4				6.9 – 32.3
Whole blood (n = 110)							
Mean	7.5	6.0%	4.3	3.5%	111.6	90.2%	124.8
Range	3.3 – 12.2		1.6 – 8.3		70.3 – 178.6		75.2 – 193.8

Petteys 2009

Observed concentrations - ARUP

Thiamine vitamer concentrations			
	DEFICIENT	**SUFFICIENT**	**INCREASED**
Whole blood [TDP] **n = 105,158**	<70 nmol/L	70 – 180 nmol/L	>180 nmol/L
	12%	81%	7%
Plasma [T] + [TMP] **n = 42,630**	<8 nmol/L	8 – 30 nmol/L	>30 nmol/L
	20%	64%	16%

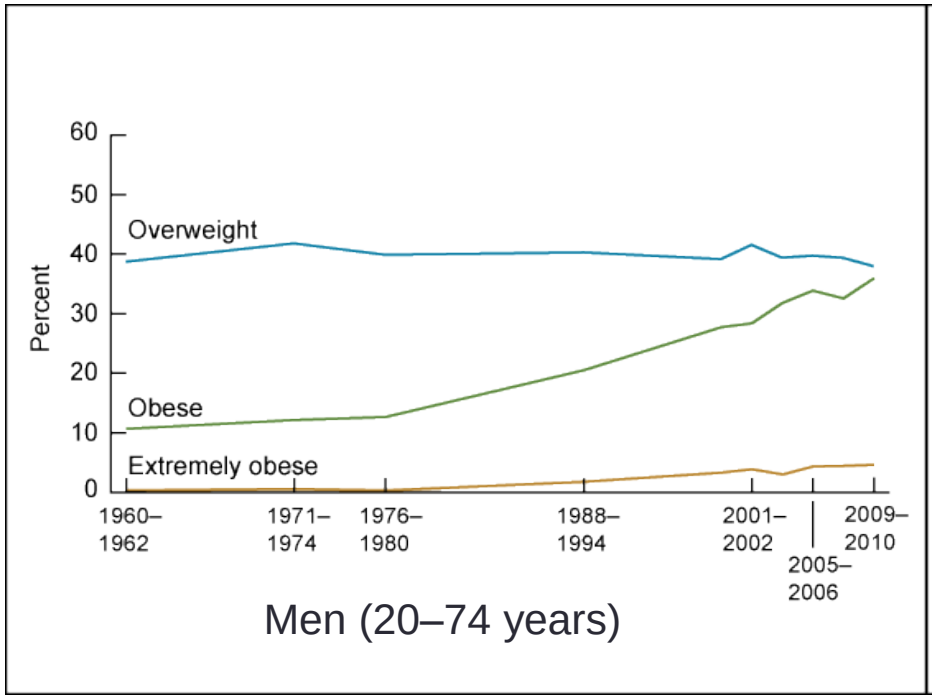
Reference intervals (RI) established using fasting specimens collected from self-reported healthy adults.

Davis 2015

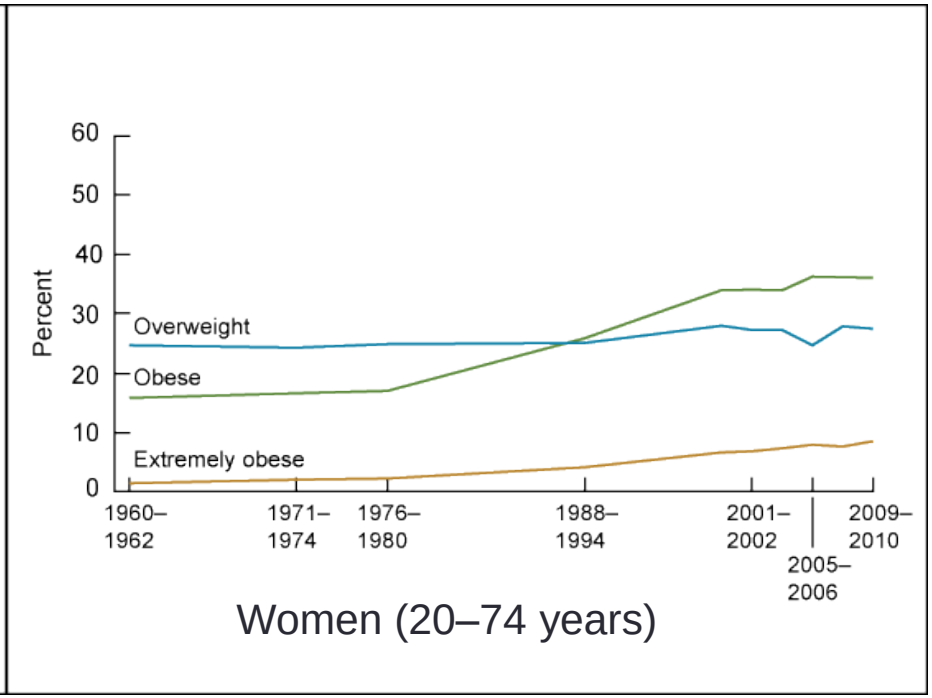
Thiamine Deficiency

- 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

Trends in overweight, obesity, and extreme obesity in adults United States, 1960–1962 through 2009–2010



Men (20–74 years)



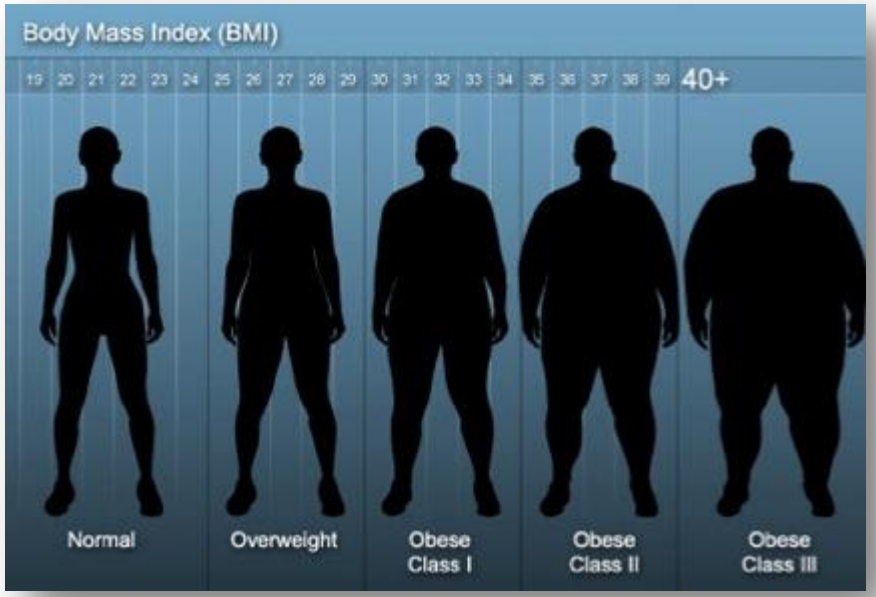
Women (20–74 years)

Overweight	BMI ≥25 kg/m^2
Obesity	BMI ≥30 kg/m^2
Extreme obesity	BMI ≥40 kg/m^2

www.cdc.gov

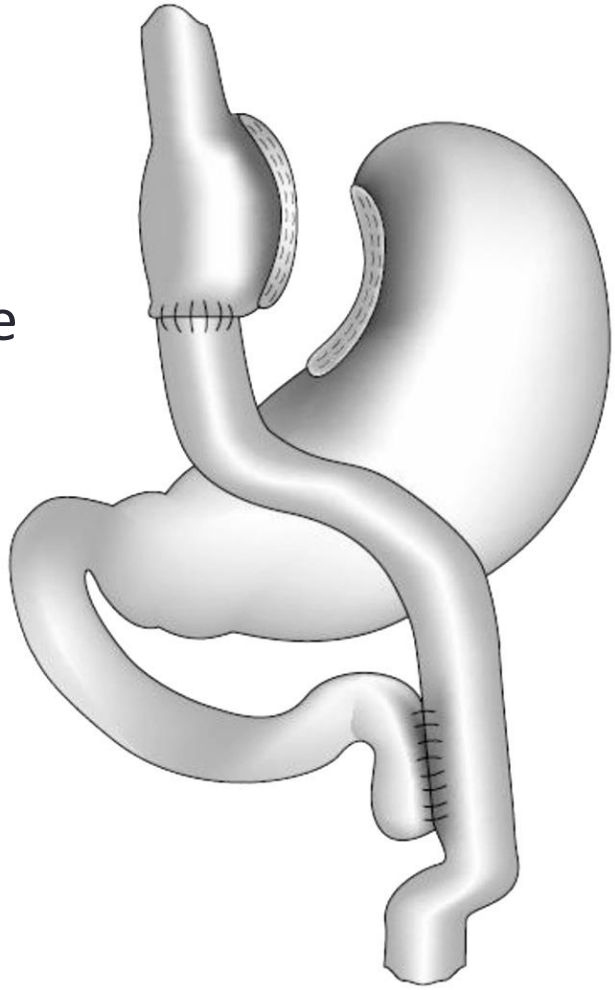
Obesity and Nutrition

- 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



Thiamine Deficiency

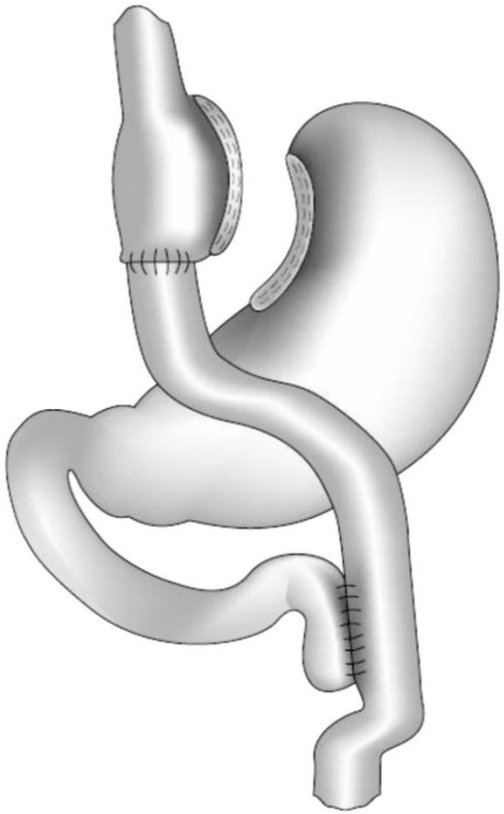
- Potential postoperative complication
- Patients may be thiamine deficient before surgery
- Thiamine requirements increased after surgery
- Early symptoms are common to many disorders
  - Fatigue
  - Irritability
  - Poor memory
  - Abdominal discomfort
  - Anorexia
  - Sleep disturbances
- Moderate 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 B_1 P 7 (8 -30 nmol/L)
  - 7/31 Vitamin B_1 P <2
  - 9/09 Vitamin B_1 P 120
  - 9/12 Vitamin B_1 WB 242 (70-180 nmol/L)
- Case 2 21F
  - 3/05 Vitamin B_1 WB 35 (70-180 nmol/L)
  - 3/11 Roux-en-Y gastric bypass surgery
  - 3/23 Difficulty ingesting food, fluids
  - 5/10 Vitamin B_1 WB 15
  - 5/25 Vitamin B_1 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