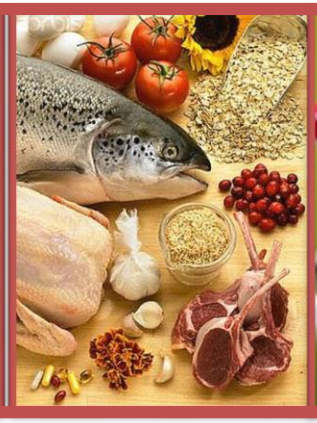
# Chapter 9 : Biochemical Assessment of Nutritional Status

# Biochemical Assessment

- Provides the most **objective** and **quantitative** data on nutritional status ( compared to anthropometric, clinical methods, and dietary)

- And detects nutrient deficits long **before** anthropometric measures are altered and clinical signs and symptoms appear

# Biochemical tests

- Static (direct) tests
- Functional (indirect) tests

# Static (direct) tests

- Based on measurement of nutrient or it's metabolite in the blood, urine, or body tissues

- E.g :
  - serum measurement of albumin, Ca, or vit A
- **Limitations :**
  - They often fail to reflect the overall nutrient status of an individual or whether the body as a whole is in a state of nutrient excess or depletion
  - E.g : serum calcium is poor indicator for body's Ca status or bone mineral content

# Functional (indirect) test

- Based on the idea that
  - “the final outcome of a nutrient deficiency and it’ biologic importance are not merely a measured level in a tissue or blood, but the failure of one or more physiologic processes that rely on that nutrient for optimal performance”

# Functional (indirect) test

E.g :

- measurement of dark adaptation (assessing vit A status)
- Urinary excretion of xanthouric acid in response to consumption of tryptophan (assesses vit B6 status)

**Drawbacks :**

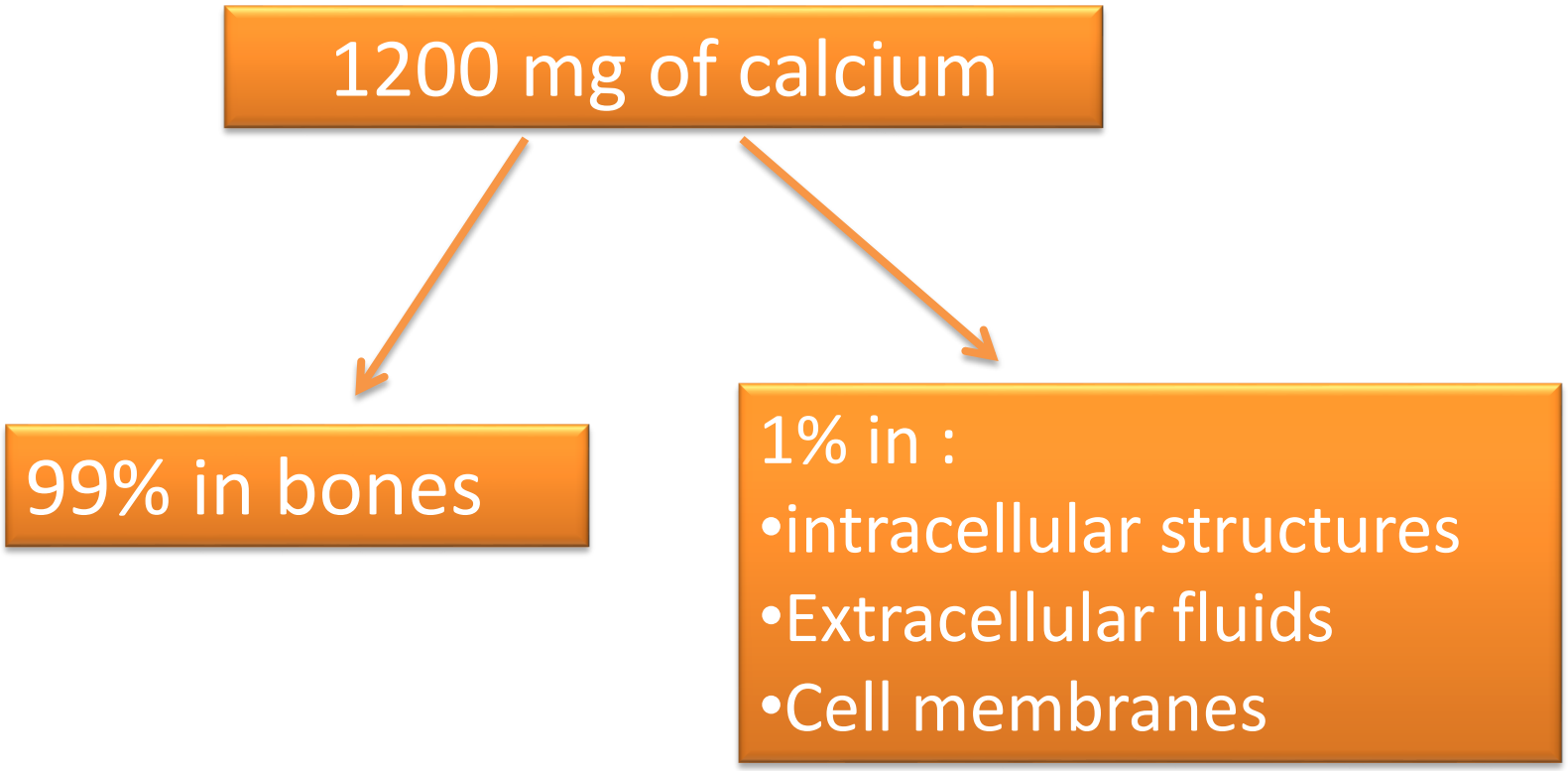
Some tests tend to be nonspecific, they may indicate general nutritional status but not allow identification of specific nutrient deficiency

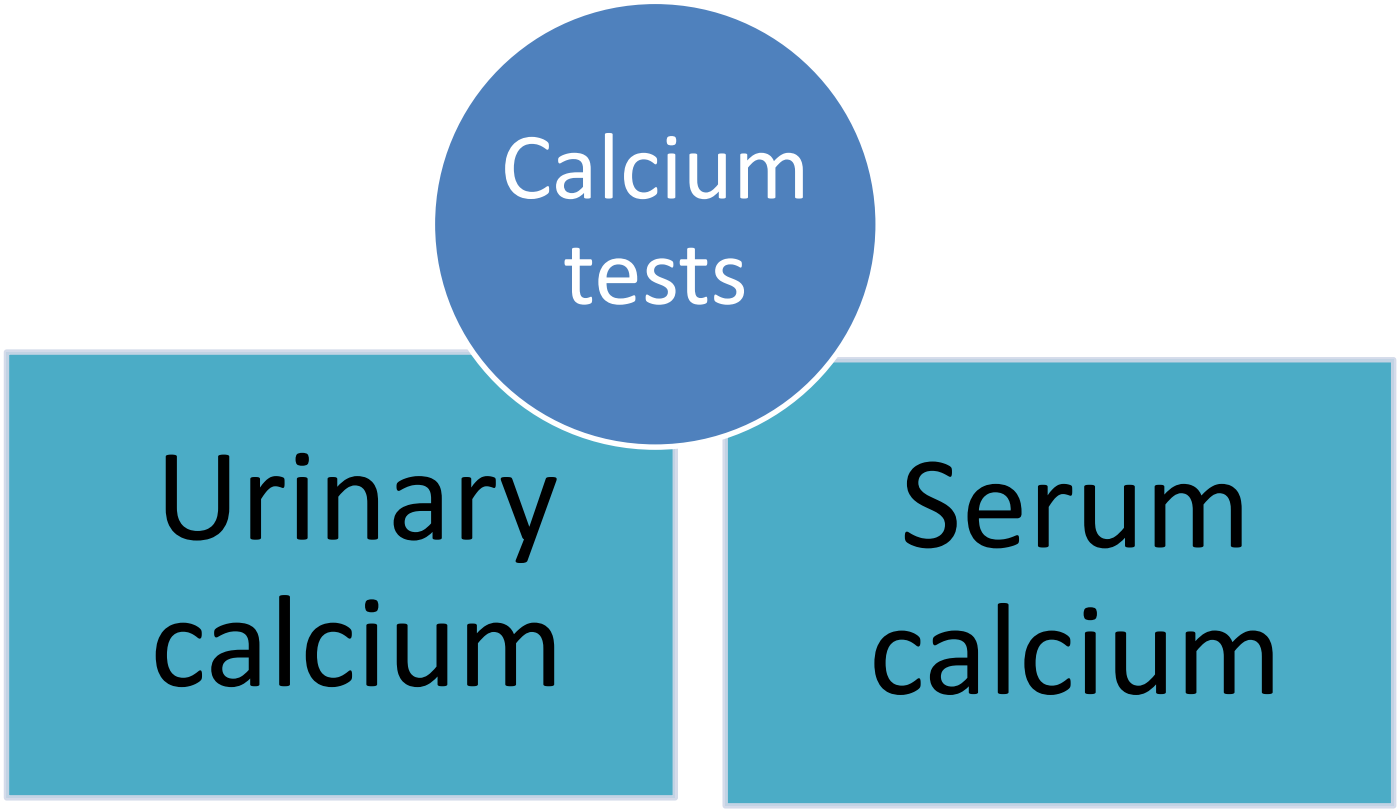
# Calcium

# Calcium functions

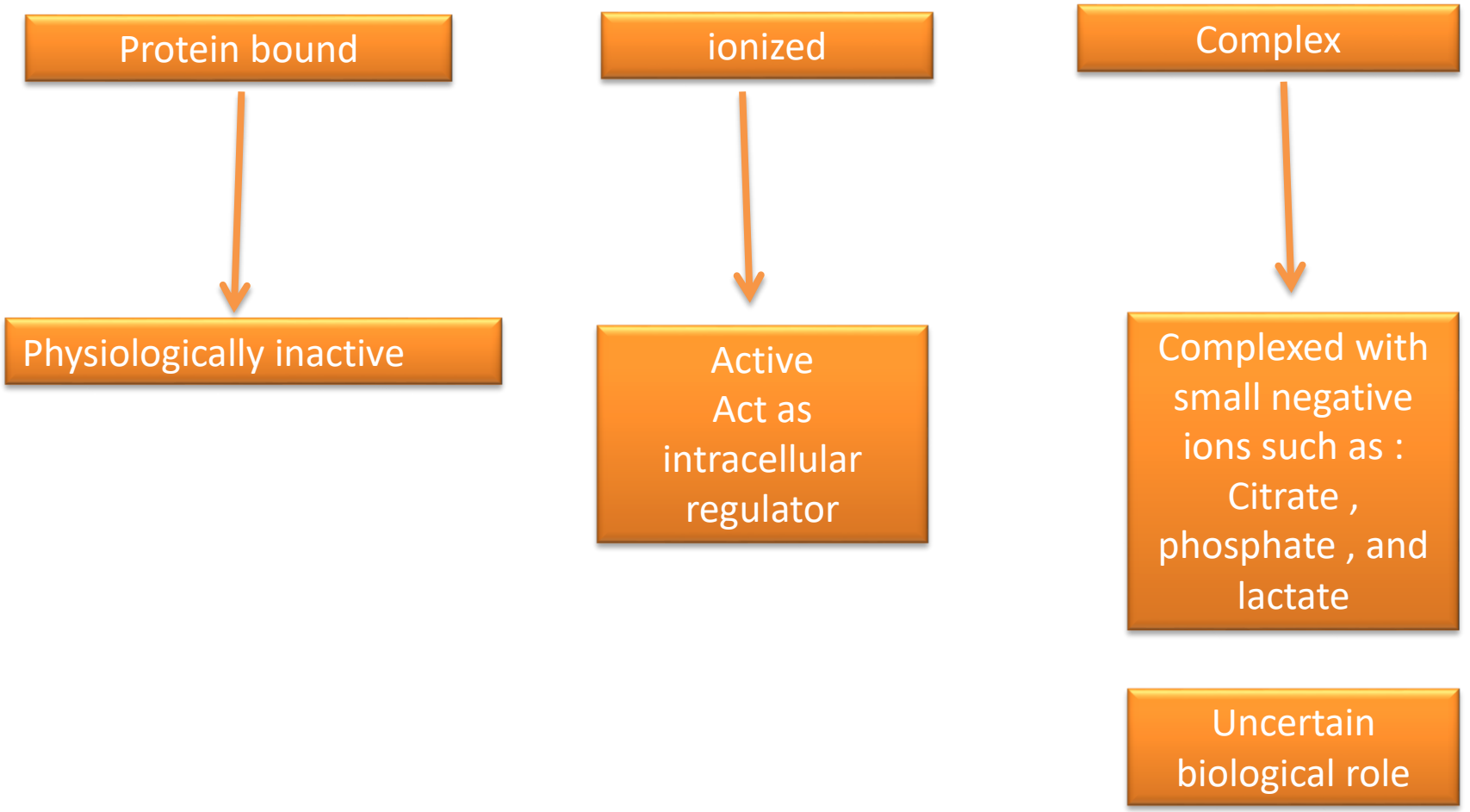
| |
|---|
| 1. Bone and tooth formation |
| 2. Muscle contraction |
| 3. Blood clotting |
| 4. Cell membrane integrity |

# Calcium in the body




Calcium tests
Urinary calcium
Serum calcium

# Serum calcium fractions




↑ levels in postmenaupausal women → marker of low bone mass

# Dietary calcium intake and serum calcium levels

Dietary calcium intake

Little association

Serum levels of Ca ; tightly controlled by the body

# Calcium Levels

## Hypocalcemia ( < 2.3 mmol/l)

- Hypoparathyroidism
- Renal disorders
- Acute pancreatitis

## Hypercalcemia ( > 2.75 mmol /l)

- ↑ intestinal absorption
- ↑ bone resorption
- ↑ renal tubular reabsorbtion from :
  - Hyperparathyrodism
  - Hyperthyrodism
  - Hyperavitaminosis D  (excessive intake of vitamin D)

# Urinary calcium

- **More responsive to changes** in dietary Ca intake than serum levels

| ↑ urinary Ca loss | ↓ urinary Ca loss |
|---|---|
| From factors leading to hypercalcemia | From factors leading to hypocalcemia |
| During day | During night |
| ↑ protein diet and ↓ in phosphate | ↑ protein diet and ↑ in phosphate |
| High urinary output | Renal failure |
| Impaired kidney's ability to reabsorb Ca | |

# Biochemical Assessment of Nutritional Status;
# **Protein Assessment**

# Introduction: Protein Status

Assessing protein status by:

1. Anthropometric
2. Biochemical
3. Clinical
4. Dietary

# Introduction: Protein Status

Biochemical Models:

1. Evaluation of **Somatic** protein

   Within skeletal muscles

2. Evaluation of **Visceral** protein

   Within organs or viscera of body, blood cells & Serum protein

# Introduction: Protein Status

**Somatic** + **Visceral**

= 30-50% of total protein

Contain <u>metabolically available protein</u>

*body cell mass*

1. **Somatic**: 75% of body cell mass

2. **Visceral**: 25% of body cell mass

# Assessing Protein Status

1. Body Weight
2. Midarm muscle circumference & muscle area
3. Creatinine Excretion & C-Height Index
4. Nitrogen balance
5. Serum protein

# Assessing Protein Status

## 2. Body Weight

Readily obtained indicator of energy & protein reserve.

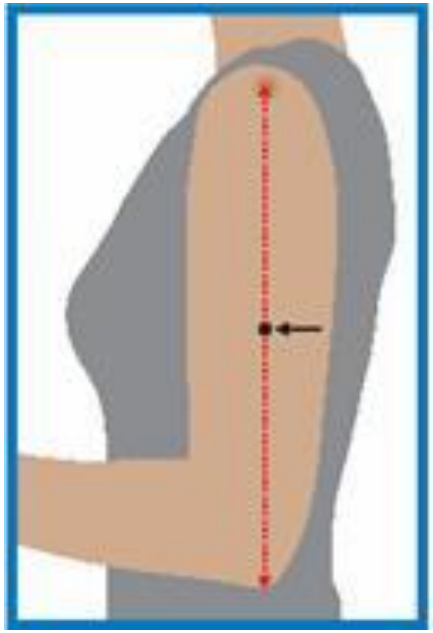
**Limitations:**

- Fail to distinguish B/N Fat & Fat free mass
- Losses can be masked by water retention

# Assessing Protein Status

3. **Midarm muscle circumference & midarm muscle area**

Assessing somatic protein status



# Creatinine Excretion & C-Height Index

24-hrs urine → estimating muscle mass

**Creatinine;**

product of skeletal muscle (excreted in a **relatively constant proportion** to **the muscle mass)**

| **Creatinine; 24 hrs urine (mg/kg) of recommended weight** | |
|---|---|
| Male | Female |
| 23 | 18 |

# C-Height Index

$$\text{CHI} = \frac{24\text{-}hr\ urine\ creatinine\ (mg) * 100}{Expected 24\text{-}hr\ urine\ creatinine}$$

- Expected 24-hr urine creatinine (table 9.1)

| CHI | |
|---|---|
| 60-80 % | Mild protein depletion |
| 40-60 % | Moderate protein depletion |
| <40 % | sever protein depletion |

# Creatinine Excretion & C-Height Index

**Limitations**

- 24- hr urine collection
- Effect of diet on creatinine excretion
  - Long term low protein consumers tend to have lower excretion
- Variability of creatinine excretion
- Use Wt-Ht tables to determine expected creatinine excretion based on sex & stature

# Nitrogen balance

**Nitrogen balance**

Nitrogen consumed = Nitrogen excreted

+ve; N intake > N Loss

-ve; N intake < N Loss

# Nitrogen balance

24-hr protein intake measurement

Estimate N losses from body

$$\boldsymbol{N_2 balance = \frac{PRO}{6.25} - UUN - 4}$$

$N_2 balance$ = Nitrogen Balance
$PRO$ = protein intake (g/24hrs)
$UUN$= UrinUrea Nitrogen (g/24hrs)
4; losses of protein from skin, stool, ….

# Nitrogen balance

**Limitations:**

- Measuring protein intake & N excretion

- Difficult to account for the unusually high nonunrine nitrogen losses seen in some patients . e.g. burns, vomiting..

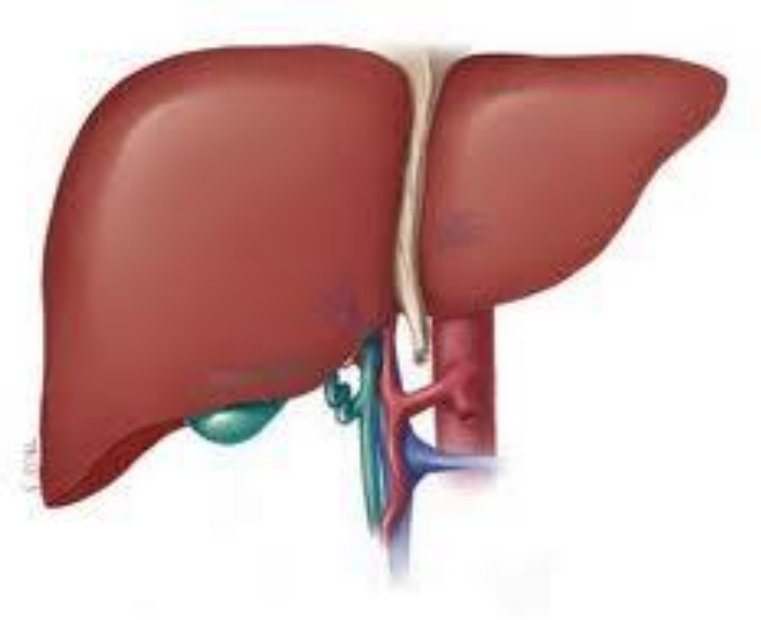
# Serum protein

Useful in:

I. Assessing protein status
II. Determine medical complication risks
III. Evaluate patient response to nutritional support

# Serum protein

- ↓ serum concentration ↔ are due to ↓ Liver production



- ↔as a consequence of ↓ Supply of a.as
- And decrease in the liver capacity to synthesize serum proteins

# Serum protein

## Albumin

| Serum Protein Used in Nutritional Assessment | | | |
|---|---|---|---|
| Serum protein | Normal Value | Half-life | Notes |
| Albumin | 45 (35-50) | 18-20 days | Poor indicator of early protein depletion and repletion (long half life) |
| NOTE | In addition to protein status, other factors affect it | | |

# Serum protein

## Transferrin

**Better index of changes in protein status compared with albumin**

| Serum Protein Used in Nutritional Assessment | | | |
|---|---|---|---|
| Serum protein | Normal Value | Half-life | Notes |
| Transferrin | 2.3 (6.2-4.3) | 8-9 days | ↑ Pregnancy & estrogen therapy & acute hepatitis<br>↓ chronic infections, uremia, and acute catabolic status |
| | | | |

# Serum protein

**Transferrin**

- Smaller half life & body pool → better index of changes in protein status than albumin

- Not for intervention: level ↓ due to many reasons

# Serum protein

## Prealbumin

| Serum Protein Used in Nutritional Assessment | | | |
|---|---|---|---|
| Serum protein | Normal Value | Half-life | NOTES |
| Prealbumin | 0.30 (0.2-0.4) | 2-3 days | ↑ Chronic renal failure & Dialysis, nephrotic syndrome<br>↓ catabolic state, after surgery, hyperthyroidism |
| | | | |

# Serum protein

**Prealbumin**

- More sensitive
- Early stages of malnutrition
- The **best** for intervention
  - Returns rapidly to the expected level (in response to adequate energy without sufficient protein intake) → not reliable to terminate the nutritional support

# Serum protein

## Retinol Binding Protein (act as a carrier for retinol)

| Serum Protein Used in Nutritional Assessment | | | |
|---|---|---|---|
| Serum protein | Normal Value | Half -life | NOTES |
| RBP | 0.372 | 12 hrs | ↑ renal disease<br>↓ vit A deficiency, catabolic state, surgery, hyperthyroidism |
| | | | |

# Serum protein

**Retinol Binding Protein**

- Retinol when complexed with prealbumin
- Respond quickly to PEM intervention
- Smaller body pool & half-life
- Like prealbumin : better indicator for **recent** dietary intake than of overall nutritional status

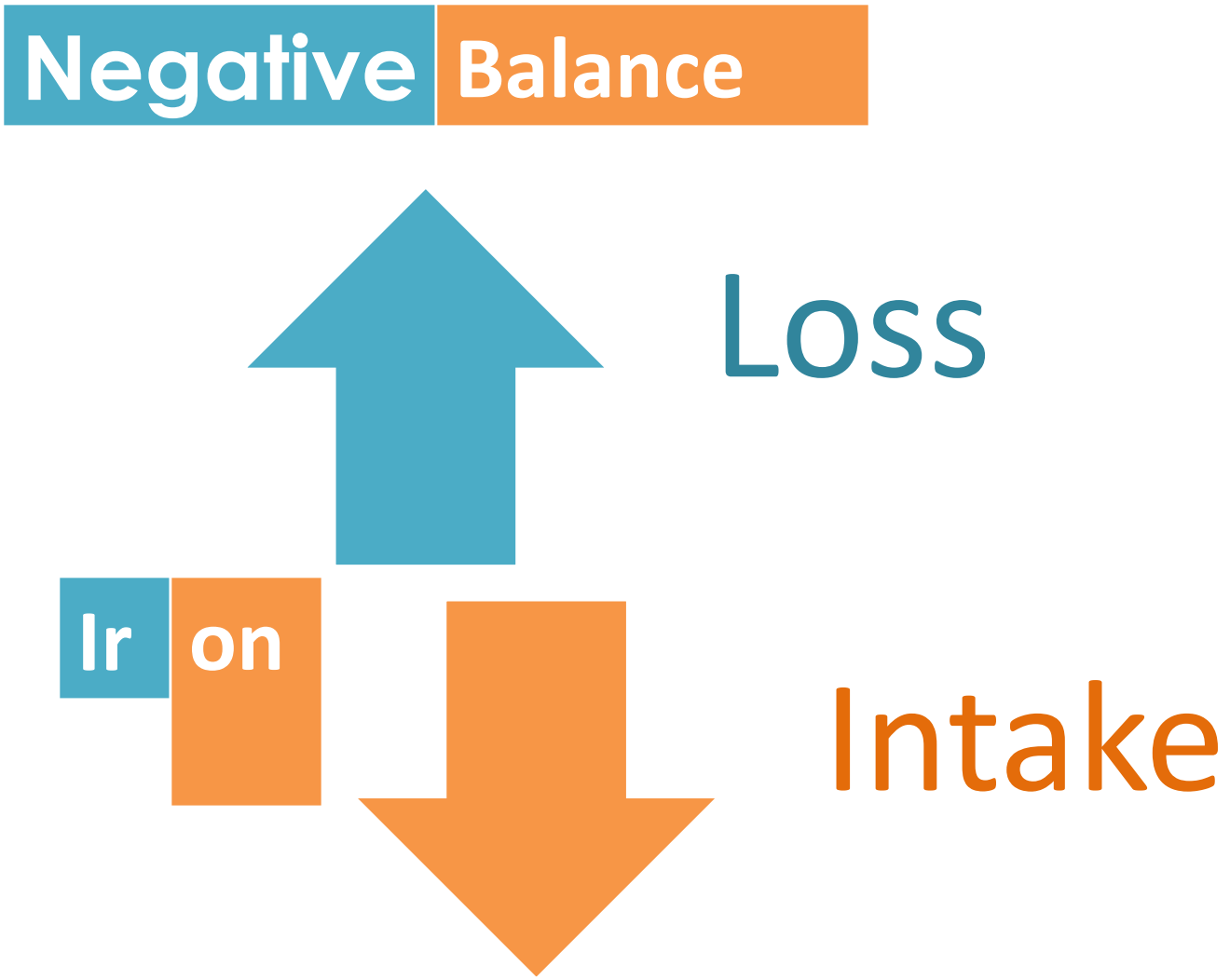
# Conclusion

Which one is the best indicator?

# IRON STATUS

*Assessment & Evaluation*

# Iron Deficiency



# What causes Iron Loss?

Heavy menstruation

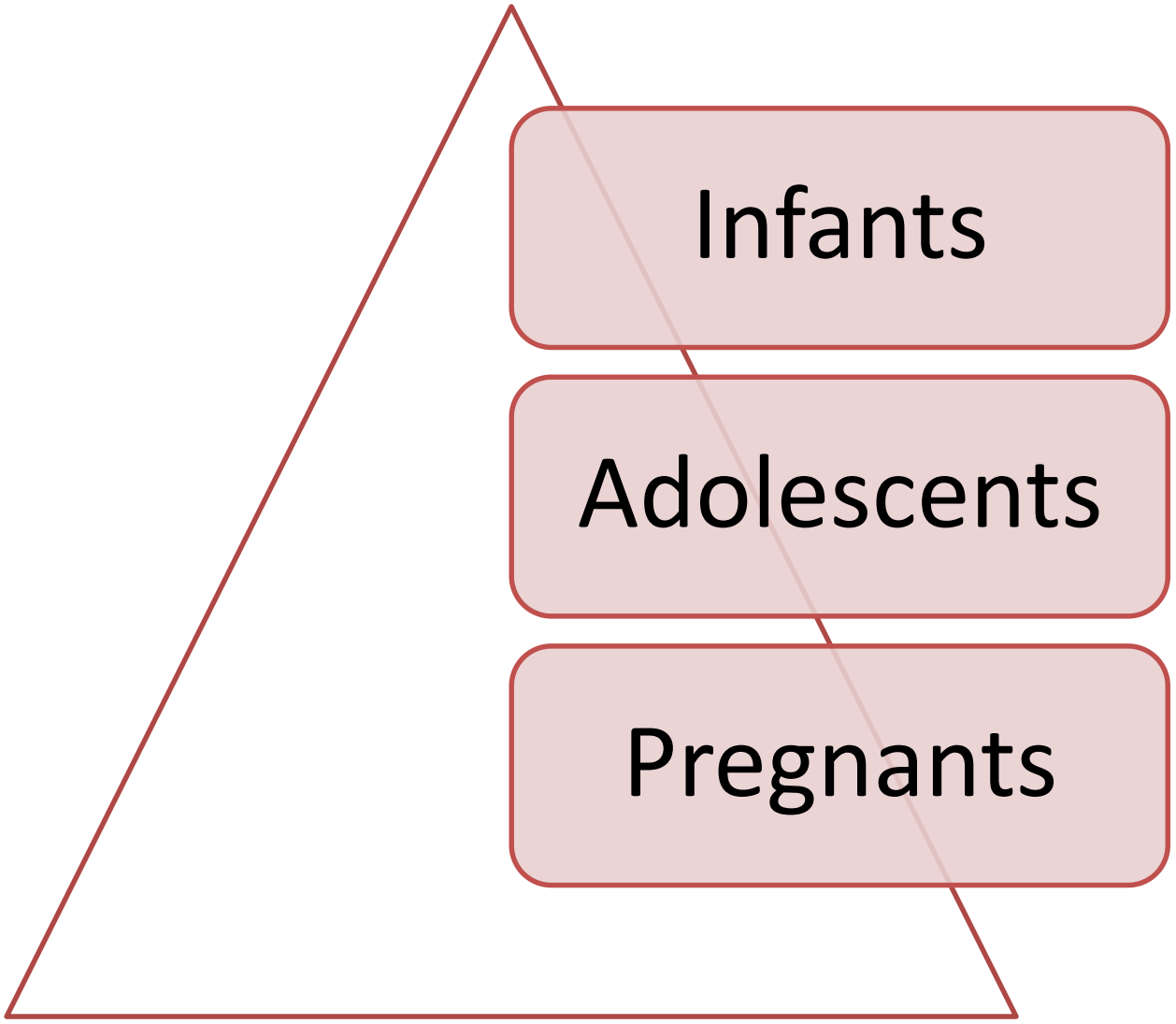
Frequent blood donation

Early feeding of cow's milk to infants

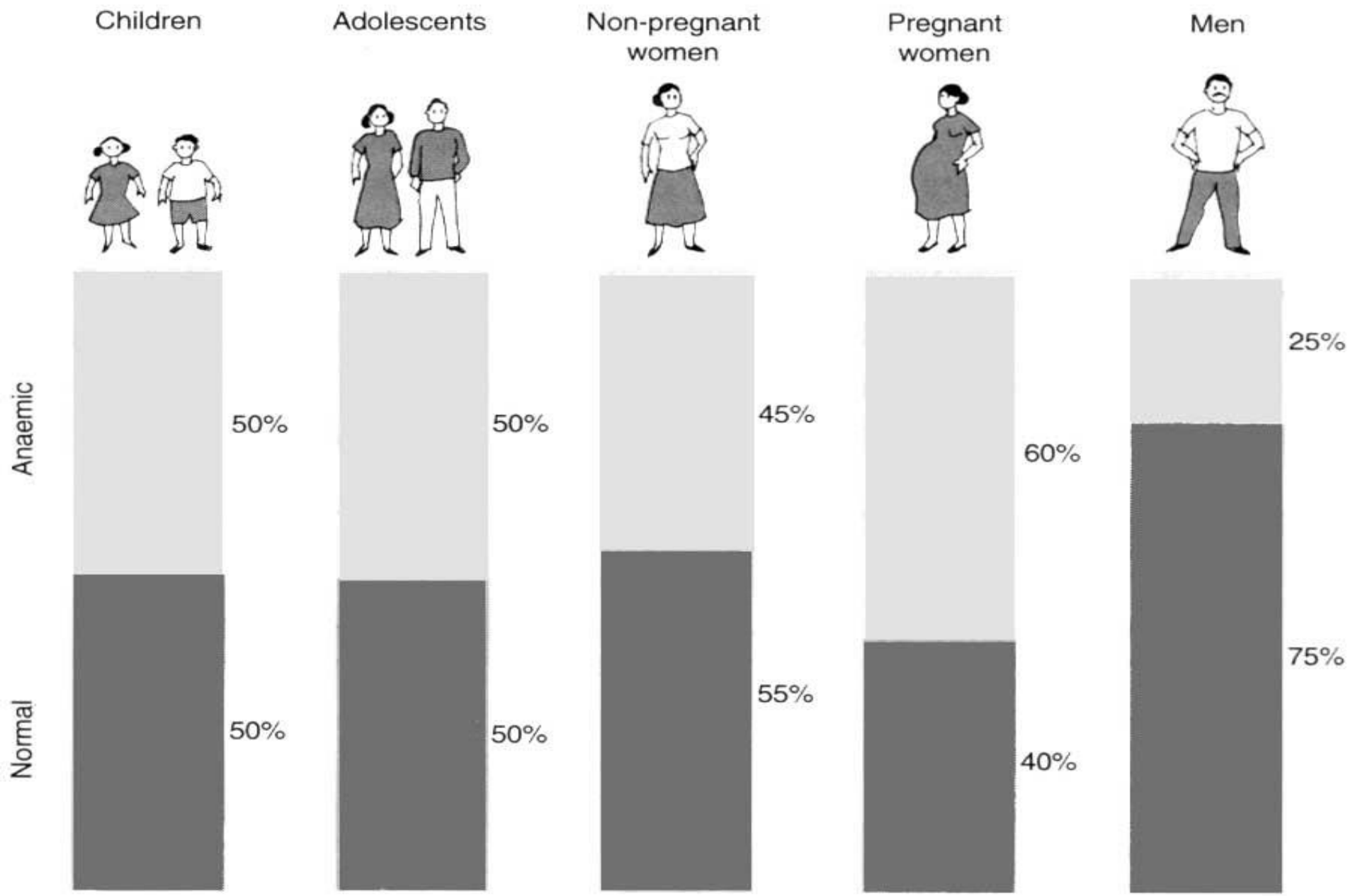
Frequent aspirin use

GI Bleeding

# Groups at risk of iron deficiency

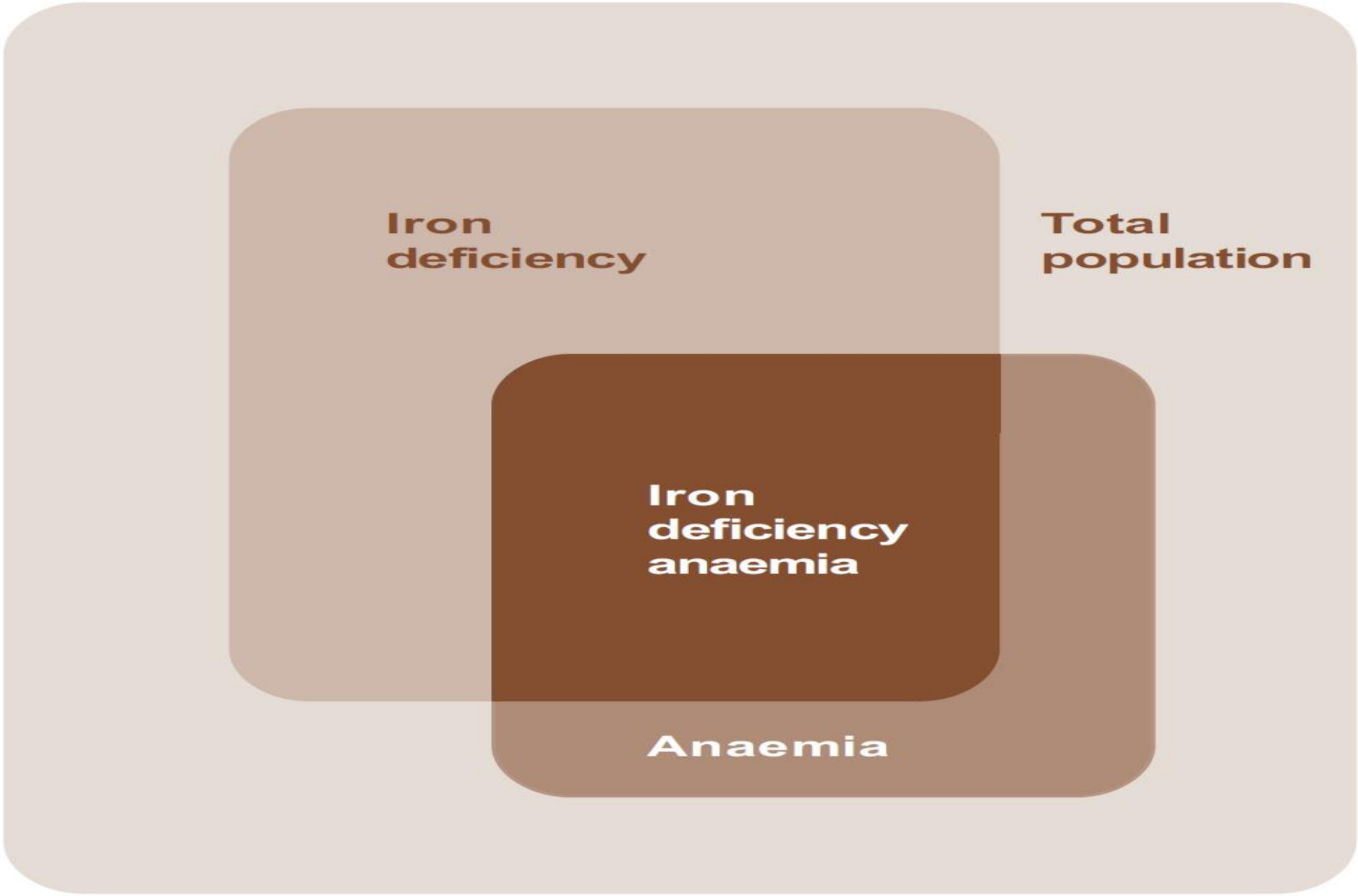


# Anemia Prevalence among the population 1.1.1



Iron deficiency

Iron-deficiency anemia




*Source:* Adapted from Yip R. Iron nutritional status defined. In: Filer IJ, ed. *Dietary Iron: birth to two years.* New York, Raven Press, 1989:19-36.

# Stages of iron deficiency (table 9.3)

| Stage | Descriptive term | Biochem. test |
|---|---|---|
| **1st** | **Depleted iron stores** | **Serum ferritin level** |
| 2nd | | Transferrin<br>tion<br>cyte<br>rphyrin |
| 3rd | Iron-deficiency anemia | Hemoglobin |
| | | Mean corpuscular volume |

Stage 1 : not associated with any adverse physiological effects

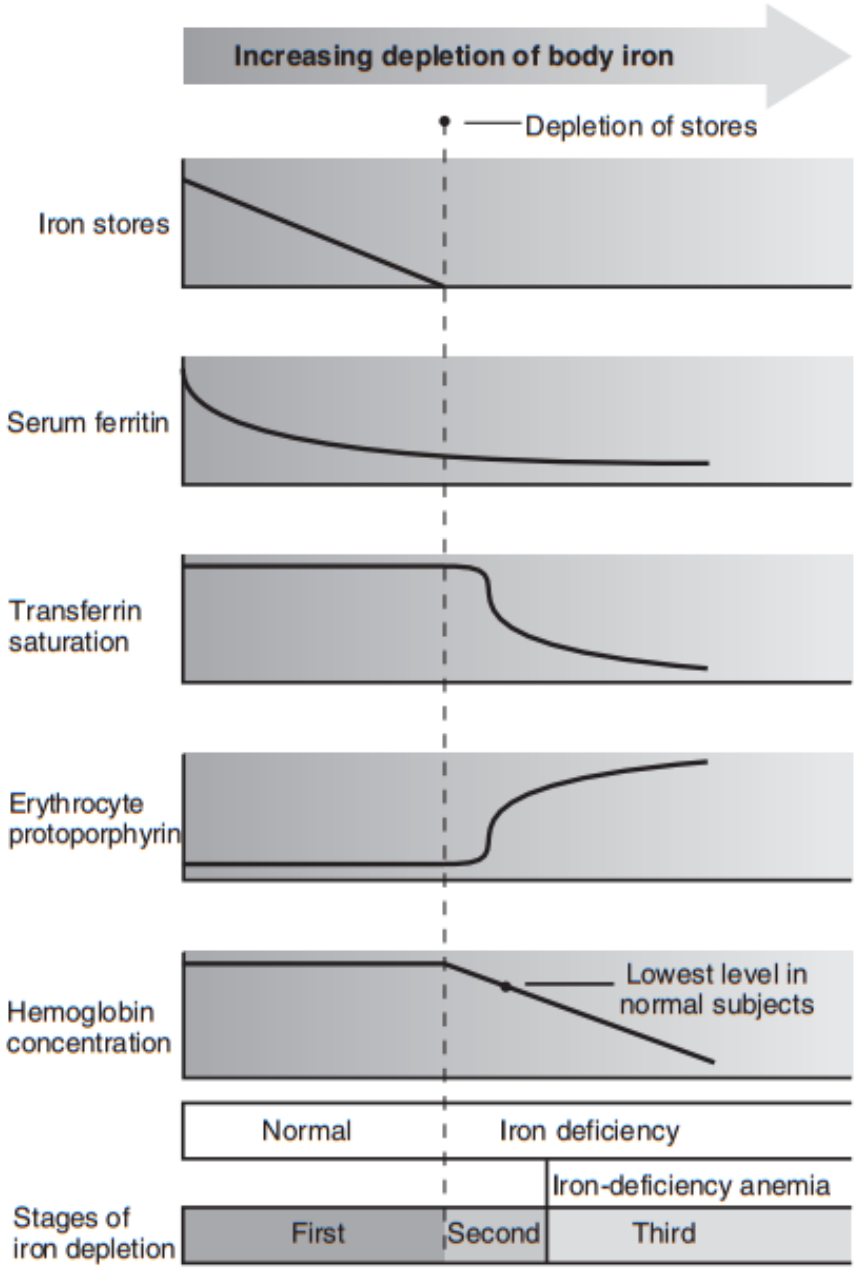



**Figure 9.1** **Changes in body iron compartments and laboratory assessments of iron status during the stages of iron depletion.**

# First Stage

**Serum ferritin**

**Primary Storage form of iron**

**Liver, Spleen & bone marrow**

# Stages of iron deficiency 1.1.2

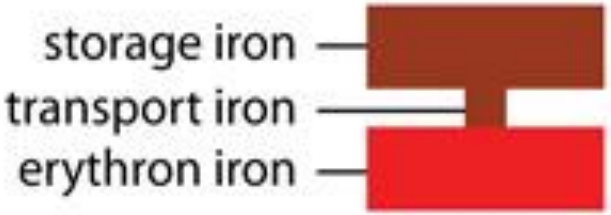

| | Normal | Iron depletion | Iron deficient erythropoiesis | Iron deficiency anemia |
|---|---|---|---|---|
| Marrow iron | 2-3+ | 0-1+ | 0 | 0 |
| Serum Fe (µg/dl) | 150 | 120 | <100 | <20 |
| % saturation | 40 | 35 | <30 | <20 |
| HCT (%) | 45 | 45 | 41 | <40 |
| RBC morphology | normal | normal | normal | microcytic hypochromic |

## (Table 9.5) Cutoff Values indicative of iron deficiency

| Age (yr) | Serum ferritin (mcg/L) |
|---|---|
| 1-2 | -------- |
| 3-4 | <10 |
| 5-10 | <10 |
| 11-14 | <10 |
| 15-74 | <12 |

# Stages of iron deficiency 1.1.2

Insufficient iron for normal production of hemoglobin




| | Normal | Iron depletion | Iron deficient erythropoiesis | Iron deficiency anemia |
|---|---|---|---|---|
| Marrow iron | 2-3+ | 0-1+ | 0 | 0 |
| Serum Fe (µg/dl) | 150 | 120 | <100 | <20 |
| % saturation | 40 | 35 | <30 | <20 |
| HCT (%) | 45 | 45 | 41 | <40 |
| RBC morphology | normal | normal | normal | microcytic hypochromic |

# Stages of iron deficiency (table 9.4)

| Stage | Descriptive term | Biochem. test |
|---|---|---|
| 1st | Depleted iron stores | Serum ferritin level |
| **2nd** | **Iron deficiency** | **Decreased Transferrin saturation** |
| | | **Increased Erythrocyte protoporphyrin** |
| 3rd | Iron-deficiency anemia | Hemoglobin |
| | | Mean corpuscular |

# Transferrin

## TRANSPORTATION OF 2 IRON ATOMS

**Storage Sites**

- Storage Sites
- Placenta
- Bone Marrow
- Enzymes

# Transferrin Saturation

Percent of transferrin that is saturated with iron

$$\textbf{TS} = \frac{\text{Serum Iron } (\mu \text{ mol/L})}{\textbf{TIBC } (\mu \text{ mol/L})} * 100$$

Measures the amount of iron capable of being bound to serum proteins

Provides an estimate of serum transferrin

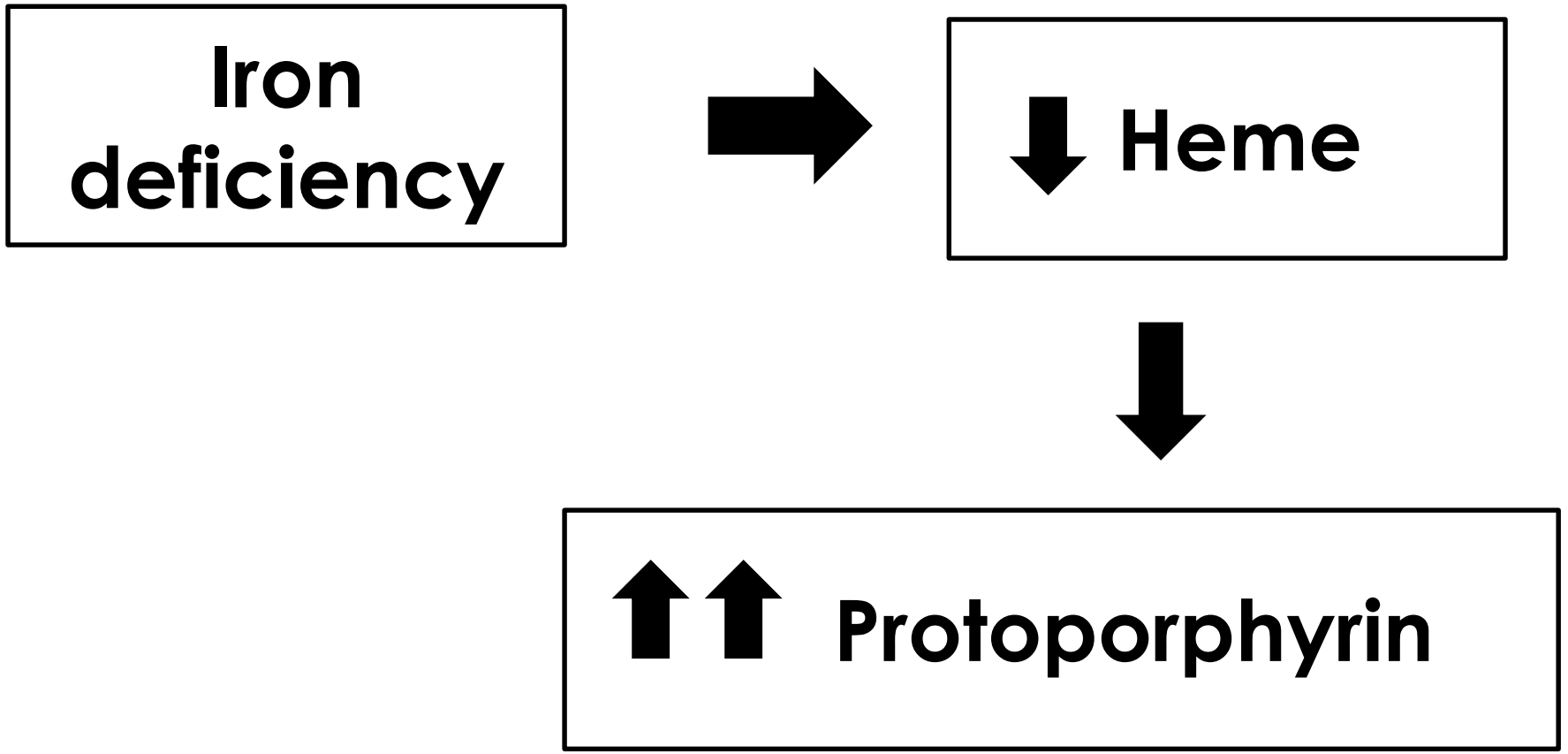
TIBC: Total Iron Binding Capacity

## (Table 9.5) Cutoff Values indicative of iron deficiency

| Age (yr) | Transferrin Saturation (%) |
|---|---|
| 1-2 | <12 |
| 3-4 | <14 |
| 5-10 | <15 |
| 11-14 | <16 |
| 15-74 | <16 |

# Protoporphyrin

**Precursor of heme**



## (Table 9.5) Cutoff Values indicative of iron deficiency

| Age (yr) | Erythrocyte Protoporphyrin (µmol/L RBC) |
|---|---|
| 1-2 | >1.42 |
| 3-4 | >1.33 |
| 5-10 | >1.24 |
| 11-14 | >1.24 |
| 15-74 | >1.24 |

# Stages of iron deficiency 1.1.2


| | Normal | Iron depletion | Iron deficient erythropoiesis | Iron deficiency anemia |
|---|---|---|---|---|
| Marrow iron | 2-3+ | 0-1+ | 0 | 0 |
| Serum Fe (µg/dl) | 150 | 120 | <100 | <20 |
| % saturation | 40 | 35 | <30 | <20 |
| HCT (%) | 45 | 45 | 41 | <40 |
| RBC morphology | normal | normal | normal | microcytic hypochromic |

# Stages of iron deficiency (table 9.4)

| Stage | Descriptive term | Biochem. test |
|---|---|---|
| 1st | Depleted iron stores | Serum ferritin level |
| 2nd | Iron deficiency | Transferrin saturation |
| | | Erythrocyte protoporphyrin |
| **3rd** | **Iron-deficiency anemia** | **Hemoglobin** |
| | | **Mean corpuscular volume** |

# Hemoglobin

Measurement of hemoglobin in whole blood is the most widely used screening test for iron-deficiency anemia

It depends on the **number of RBCs**

# Hemoglobin

| Gender | Reference value |
| --- | --- |
| Men | 140-180 g/L |
| Women | 120-160 g/L |

# Hematocrit

Percentage of RBCs making up the entire volume of whole blood

It depends on the number of RBCs

| Gender | Reference value |
|---|---|
| Men | 40-54 % |
| Women | 37-47 % |

# Hematocrit

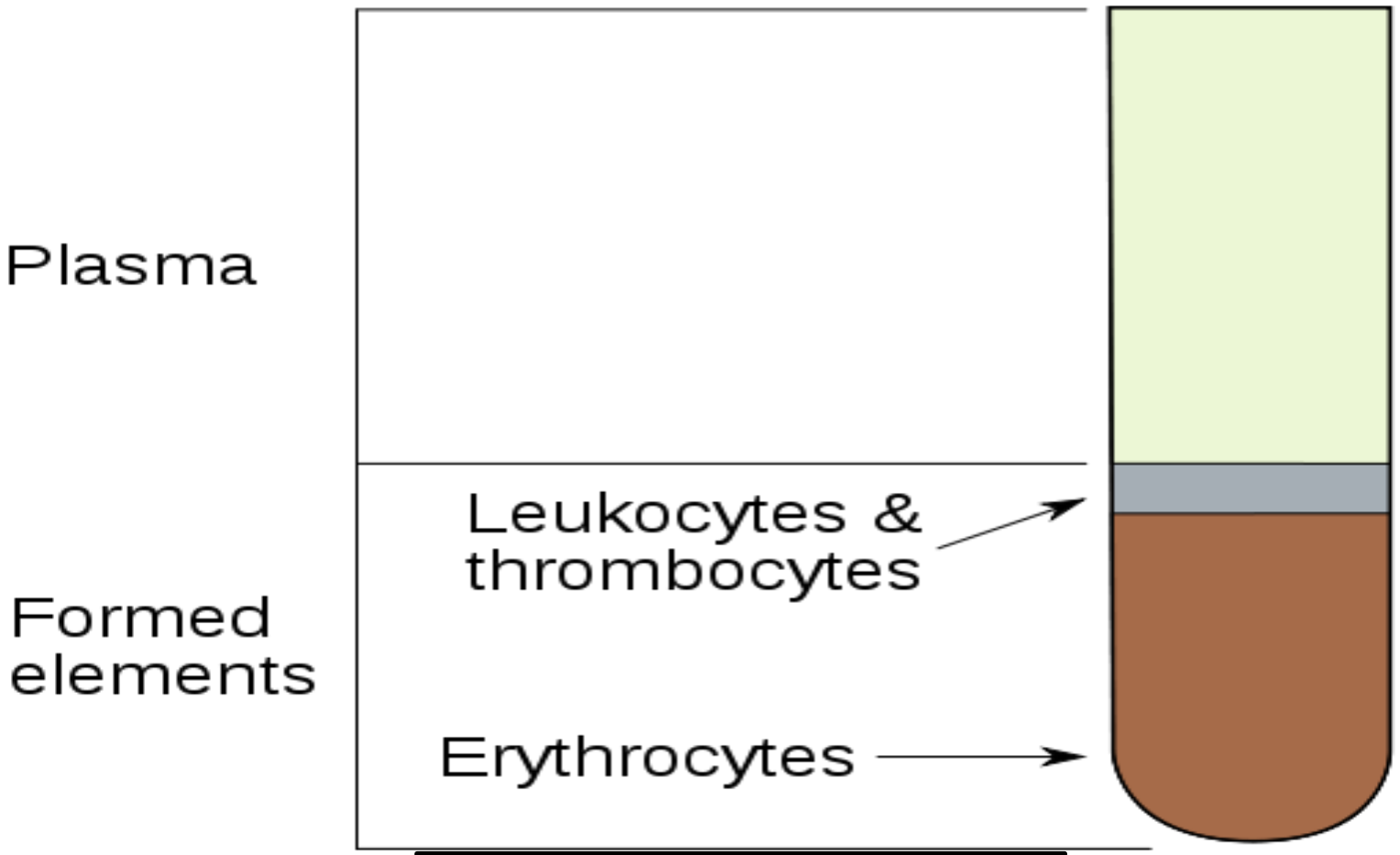



**Figure H.1**

Hematocrit can be measured manually by comparing the height of whole blood in a capillary tube with the height of RBC column after the tube is centrifuged

**Hemoglobin** **Hematocrit**

Are *not* indicators of an early iron deficiency

figure 9.1 page 323

# Stages of iron deficiency (table 9.4)

| Stage | Descriptive term | Biochem. test |
|---|---|---|
| 1st | Depleted iron stores | Serum ferritin level |
| 2nd | Iron deficiency | Transferrin saturation |
| | | Erythrocyte protoporphyrin |
| **3rd** | **Iron-deficiency anemia** | **Hemoglobin** |
| | | **Mean corpuscular volume** |

# Mean Corpuscular Hemoglobin

Amount of HG in RBCs

$$\textbf{MCH (pg)} = \frac{\text{HG level}}{\text{RBCs count}}$$

Reference value: 26 – 34 pg

It depends on the size of RBCs

# Mean Corpuscular Hemoglobin Concentration

$$\textbf{MCHC (g/L)} = \frac{\text{HG value}}{\text{Hematocrit}}$$

Reference value: 320 – 360 g/L

# Mean Corpuscular Volume

Volume of the average RBC

$$\textbf{MCV (fL)} = \frac{\text{Hematocrit}}{\text{RBC count}}$$

Reference value: 80 – 100 fL

# Factors affecting MCV

| Macrocytosis (increasing MCV) |
| --- |
| Deficiency of folate |
| Deficiency of B12 |
| Chronic liver disease |
| Alcoholism |
| Cytotoxic chemotherapy |

| Microcytosis (decreasing MCV) |
| --- |
| Chronic iron deficiency |
| Thalassemia |
| Anemia of chronic disease |
| Lead poisoning |

## (Table 9.5) Cutoff Values indicative of iron deficiency

| Age (yr) | MCV (fL) |
|---|---|
| 1-2 | <73 |
| 3-4 | <75 |
| 5-10 | <76 |
| 11-14 | <78 |
| 15-74 | <80 |

# Laboratory Measurements Used in 4 Models for Assessing Iron Deficiency (table 9.6)

| Model | Measurement Used |
| --- | --- |
| Ferritin model | Serum ferritin<br>Transferrin saturation<br>Erythrocyte protoporphyrin |
| Mean corpuscular volume (MCV) model | MCV<br>Transferrin saturation<br>Erythrocyte protoporphyrin |
| Body iron model | Soluble transferrin receptor<br>Serum ferritin |

# Ferritin Model

2 out of 3 tests should be abnormal

Overestimation of iron deficiency**

Identifying persons in the 2nd & 3rd stages of iron depletion

# MCV Model

2 out of 3 tests should be abnormal

Better than Ferritin model

Identifying persons in the 2nd & 3rd stages of iron depletion

**Ferritin Model** | **MCV Model**

Cannot distinguish iron-deficiency anemia from other causes of anemia

Because they include erythrocyte protroporphyrin as a variable

# Summary

The body iron model is considered superior because :

it is less affected by inflammation

it is only two tests

Anemia could be caused by iron deficiency or by inflammation

# FOLATE STATUS

*Assessment & Evaluation*

## Folate main function

- Coenzyme in a.a metabolism and nucleic acid synthesis
- Purine and pyrimidine synthesis

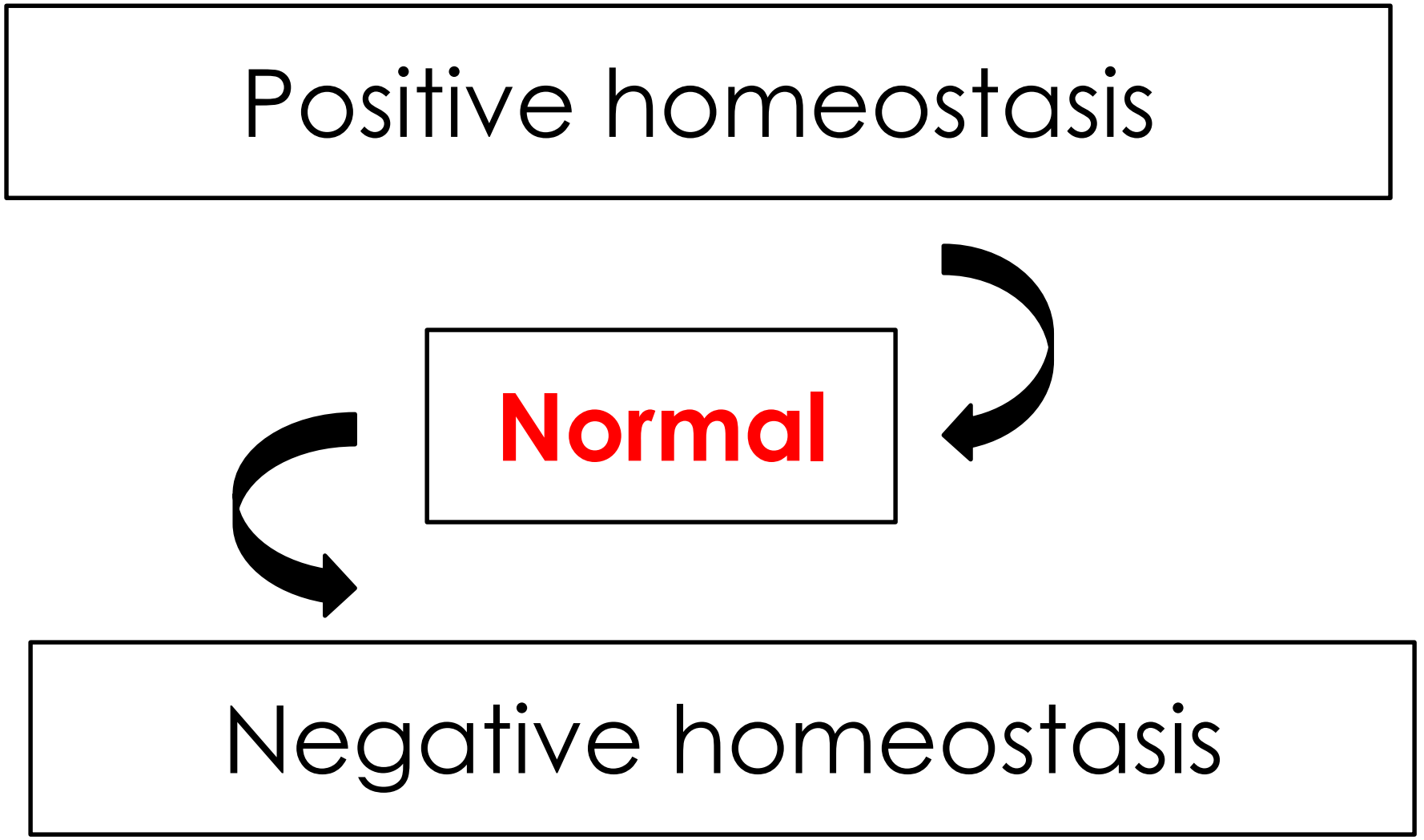
## Folate deficiency

- Inhibition of DNA synthesis
- Alteration in protein synthesis

# Clinical Features of folate deficiency

- Spina bifida
- Encephalocele
- Anencephaly

# Stages of folate deficiency



# Stages of folate deficiency

## Normal

| Table 1.1 | Normal |
| --- | --- |
| Serum folate (ng/ml) | > 5 |
| RBC folate (ng/ml) | > 200 |

# Stages of folate deficiency

## Positive homeostasis

| Table 1.2 | Early positive | Excess |
|---|---|---|
| Serum folate (ng/ml) | > 10 | > 10 |
| RBC folate (ng/ml) | > 300 | > 400 |

# Stages of folate deficiency

## Negative homeostasis

| Stage I | Stage II | Stage III | Stage IIII |
|---|---|---|---|
| Serum folate < 3 ng/ml | RBC folate < 160 ng/ml | Lobe average < 3.5<br><br>Liver folate < 1.2 µg/g | Change in RBC morphology<br><br>⬆MCV<br><br>⬇ HG |

# Assessment of folate deficiency

## Serum Folate

## Erythrocyte folate

## Deoxyuridine Suppression Test

**Serum Folate** **< 3 ng/L**

Fails to differentiate between the chronic and the transient folate deficiency

**Alcohol**

**Smoking**

**Contraceptives**

## Erythrocyte folate

**< 160 ng/L**

Best clinical index of depleted tissue stores

Unlike serum folate, it is **less subject to transient fluctuations in dietary intake**

# B12 STATUS

*Assessment & Evaluation*

# B 12

| Deficiency Symptoms |
| --- |
| Megaloblastic anemia |
| Nerve degradation |
| |

| The etiology of deficiency |
| --- |
| Vegans |
| Pernicious anemia (inadequate production of intrinsic factors) → 95% of cases |

# Stages of B12 deficiency

## Normal

| Table 1.1 | Normal |
| --- | --- |
| Holohap (pg/ml)<br><br>The transport protein of absorbed B12 | > 180 |

# Stages of B 12 deficiency

## Positive homeostasis

| Table A | Early positive | Excess |
|---|---|---|
| Holohap (pg/ml) | > 400 | > 500 |

# Stages of B12 deficiency

## Negative homeostasis

| Stage I | Stage II | Stage III | Stage IIII |
|---|---|---|---|
| HoloTC II (pg/ml) < 40 | Holohap (pg/ml)<150 | RBC folate (ng/ml) < 140 | Change in RBC morphology<br>⬆MCV<br>⬇ HG |

# Assessment of B12 deficiency

## Schilling Test

Oral dose of labeled B12

⬇

IM injection of non labeled B12

➡

Collection of 24 h urine

⬆

Amount collected (labeled) is proportional to the amount absorbed

# Assessment of B12 deficiency

## Schilling Test

In pernicious anemia , the content of the administered dose of labeled B12 should be high in the urine specimen (since the body cannot absorb it due to lack of intrinsic factor)