Chapter 9 : Biochemical Assessment of Nutritional Status

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Biochemical Assessment

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

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

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Biochemical tests

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

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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 <u>whether the body as a whole</u> 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

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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”

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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

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Calcium

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Calcium functions

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

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Calcium in the body

1200 mg of calcium

99% in bones

1% in :

- intracellular structures
- Extracellular fluids
- Cell membranes

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Calcium tests

Urinary calcium

Serum calcium

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Serum calcium fractions

Protein bound

Physiologically inactive

ionized

Active
Act as
intracellular
regulator

Complex

Complexed with small negative ions such as : Citrate , phosphate , and lactate

Uncertain biological role

↑ levels in postmenaupausal women → marker of low bone mass

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Dietary calcium intake and serum calcium levels

Little association

Dietary calcium intake ⟷ Serum levels of Ca ; tightly controlled by the body

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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)

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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	

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Biochemical Assessment of Nutritional Status;
Protein Assessment

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Introduction: Protein Status

Assessing protein status by:

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

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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

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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

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Assessing Protein Status

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

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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

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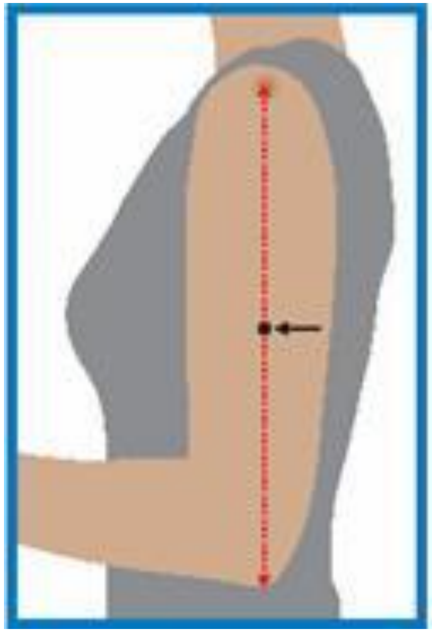
Assessing Protein Status

3. Midarm muscle circumference & midarm muscle area

Assessing somatic protein status



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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

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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

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Creatine 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

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Nitrogen balance

Nitrogen balance

Nitrogen consumed = Nitrogen excreted

+ve; N intake > N Loss

-ve; N intake < N Loss

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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, ….

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Nitrogen balance

Limitations:

- Measuring protein intake & N excretion

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

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Serum protein

Useful in:

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

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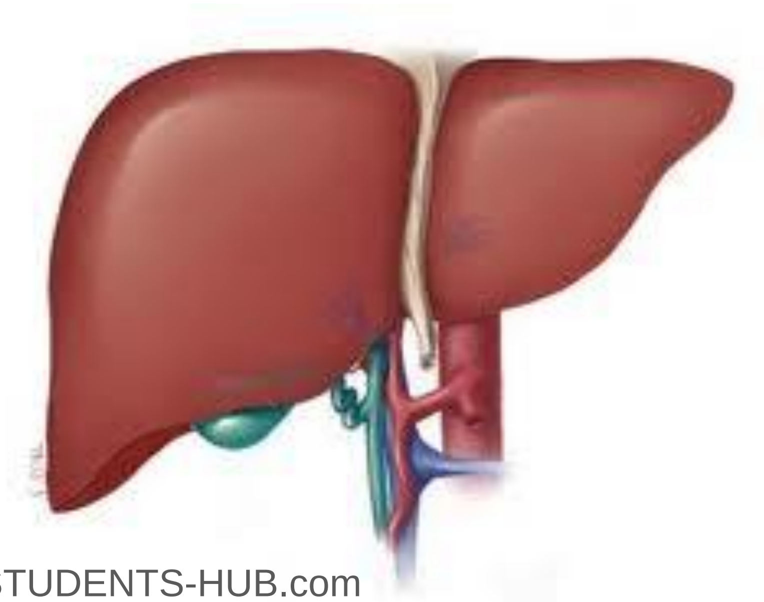
Serum protein

- ↓ 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

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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		

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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 ↓ chronic infections, uremia, and acute catabolic status

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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

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Serum protein

Prealbumin

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

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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

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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 ↓ vit A deficiency, catabolic state, surgery, hyperthyroidism

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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

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Conclusion

Which one is the best indicator?

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IRON STATUS

Assessment & Evaluation

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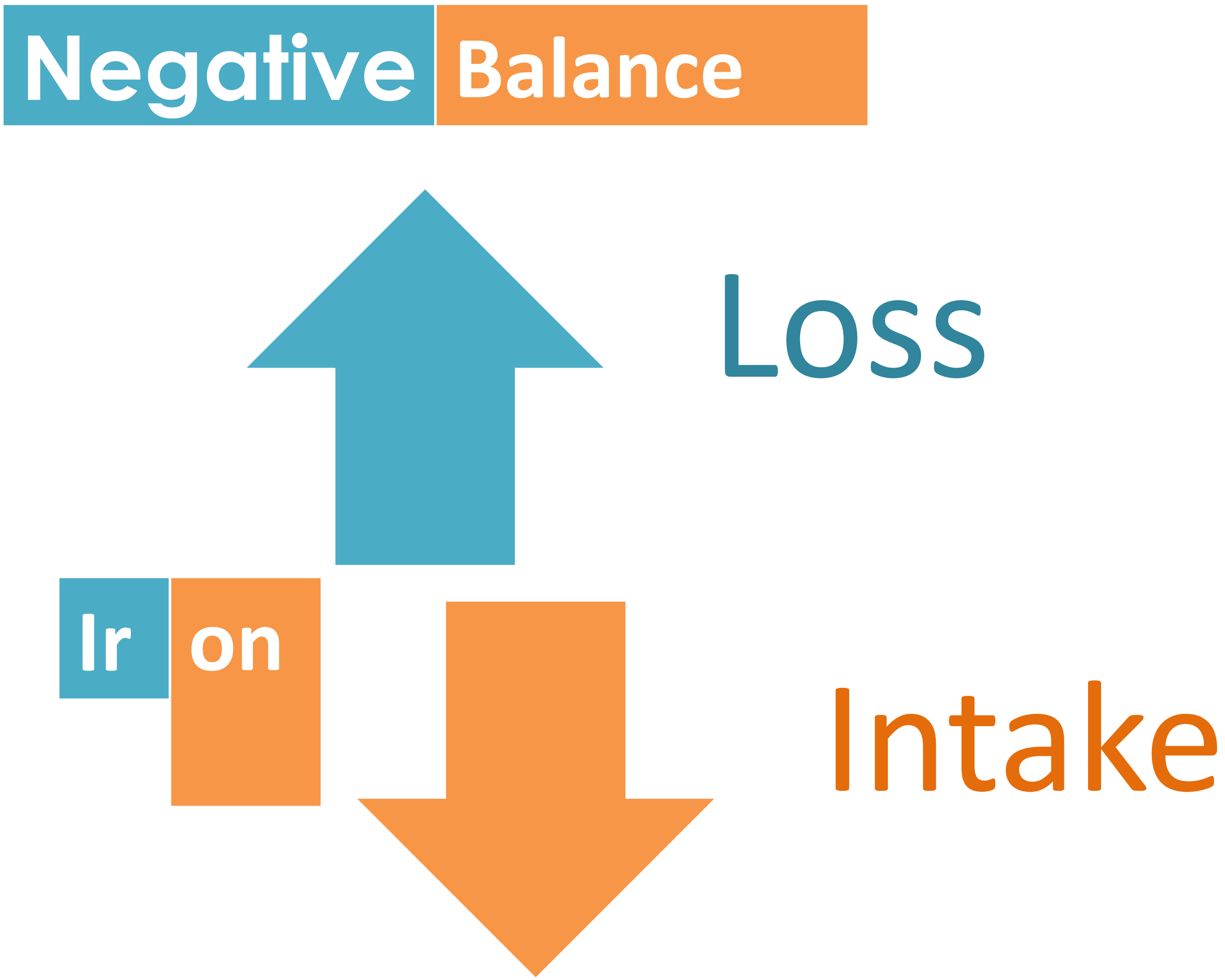
Iron Deficiency



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What causes Iron Loss?

Heavy menstruation

Frequent blood donation

Early feeding of cow's milk to infants

Frequent aspirin use

GI Bleeding

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Groups at risk of iron deficiency

- Infants
- Adolescents
- Pregnants

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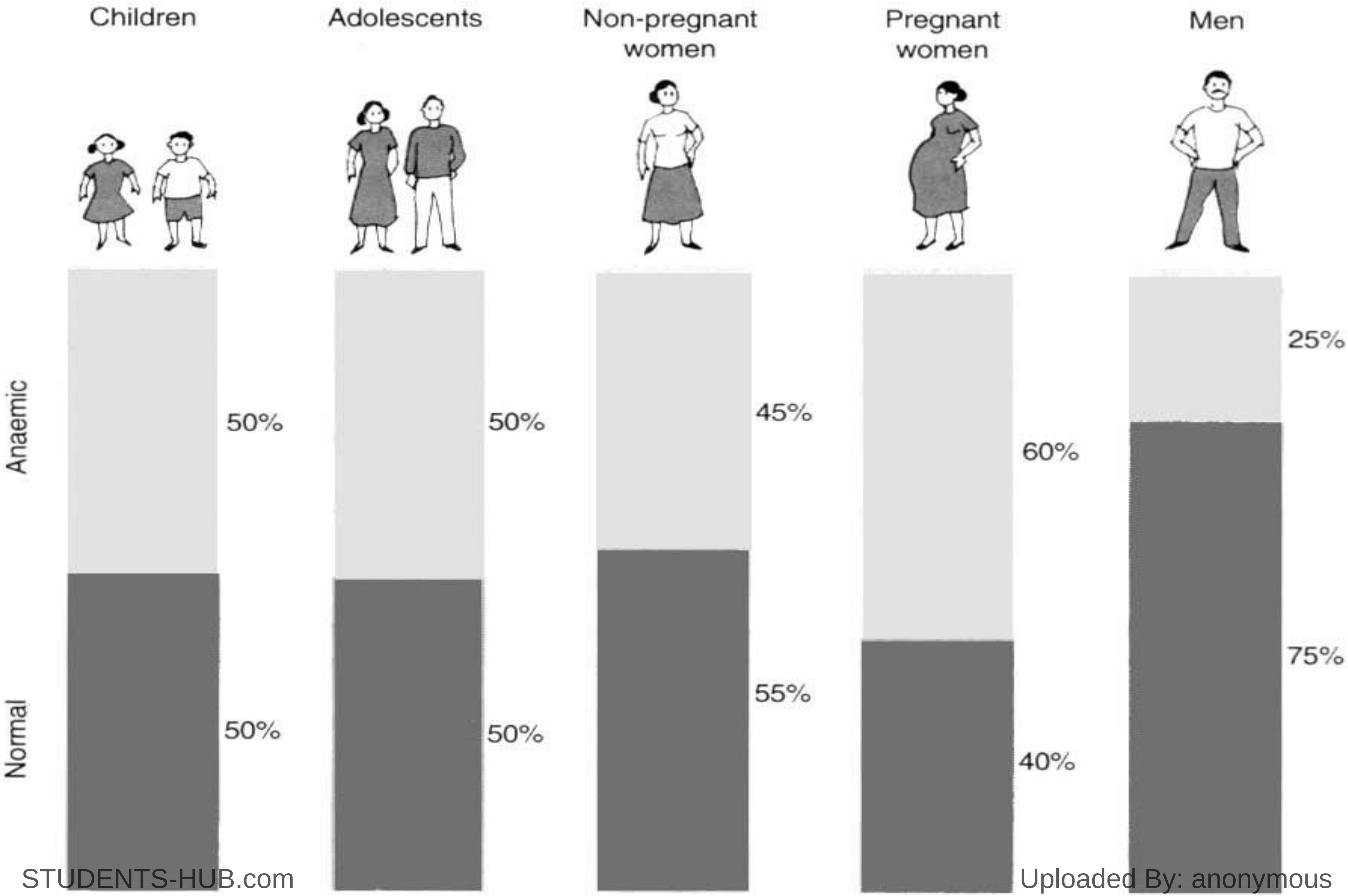
Anemia Prevalence among the population 1.1.1



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Iron deficiency

↓

Iron-deficiency anemia

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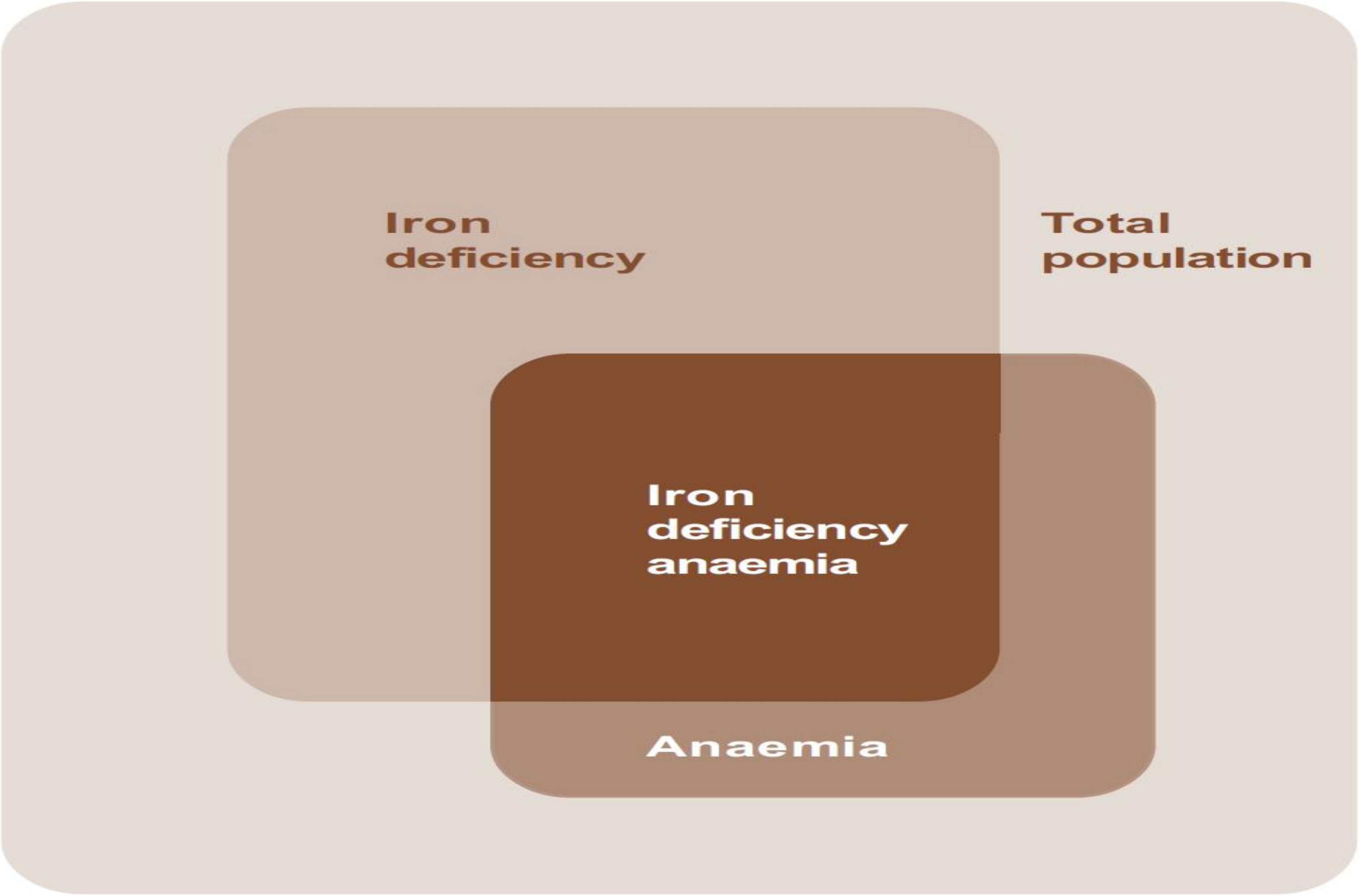

Source: Adapted from Yip R. Iron nutritional status defined. In: Filer IJ, ed. *Dietary iron: birth to two years.* New York, Raven Press, 1989:19–36.

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Stages of iron deficiency (table 9.3)

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

Stage 1 : not associated with any adverse physiological effects

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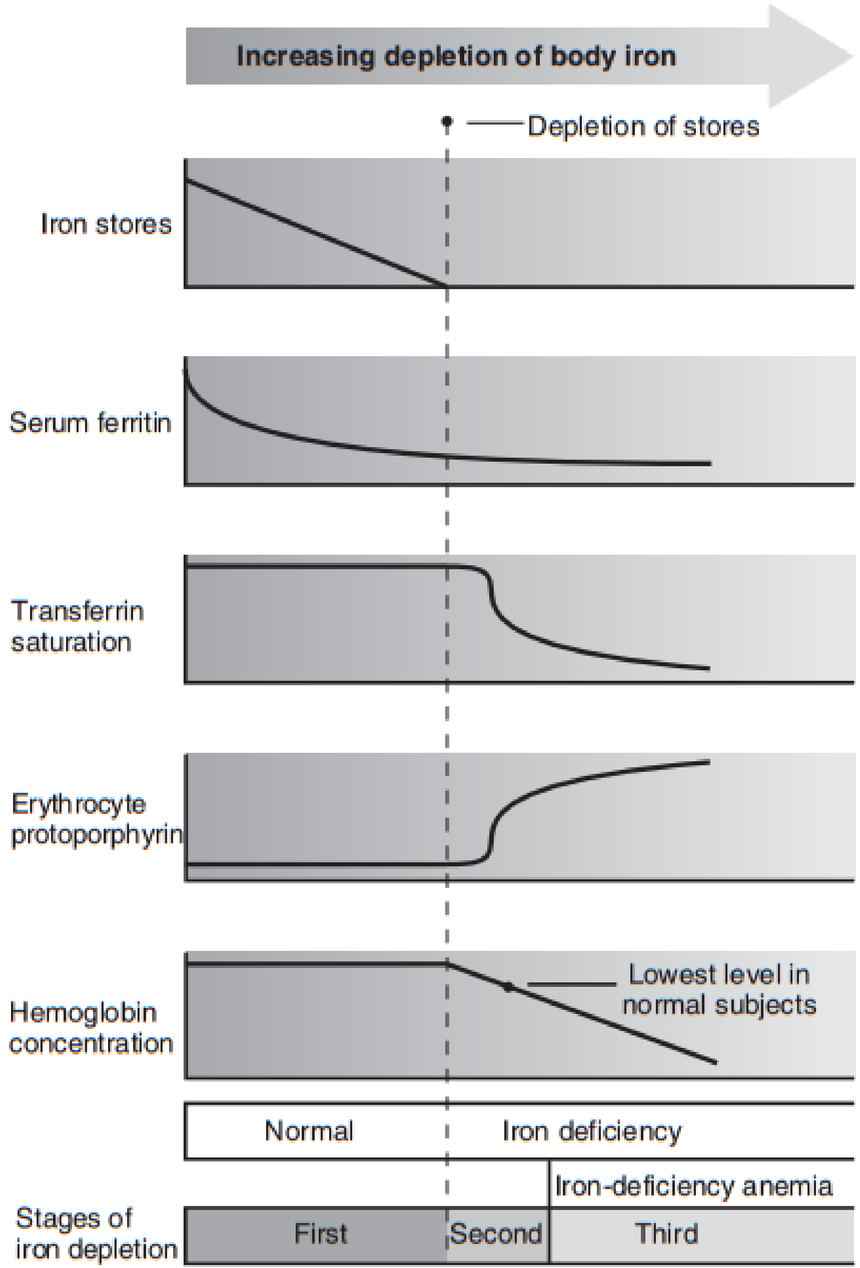

Figure 9.1 Changes in body iron compartments and laboratory assessments of iron status during the stages of iron depletion.

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First Stage

Serum ferritin

Primary Storage form of iron

Liver, Spleen & bone marrow

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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

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(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

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Stages of iron deficiency 1.1.2

Insufficient iron for normal production of hemoglobin

	Normal	Iron depletion	Iron deficient erythropoiesis	Iron deficiency anemia

	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

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Stages of iron deficiency (table 9.4)

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

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Transferrin

TRANSPORTATION OF 2 IRON ATOMS

Storage Sites

↓

Storage Sites
Placenta
Bone Marrow
Enzymes

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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

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(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

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Protoporphyrin

Precursor of heme

Iron deficiency → ↓ Heme → ↑↑ Protoporphyrin

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(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

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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
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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

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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**

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Hemoglobin

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

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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 %

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Hematocrit

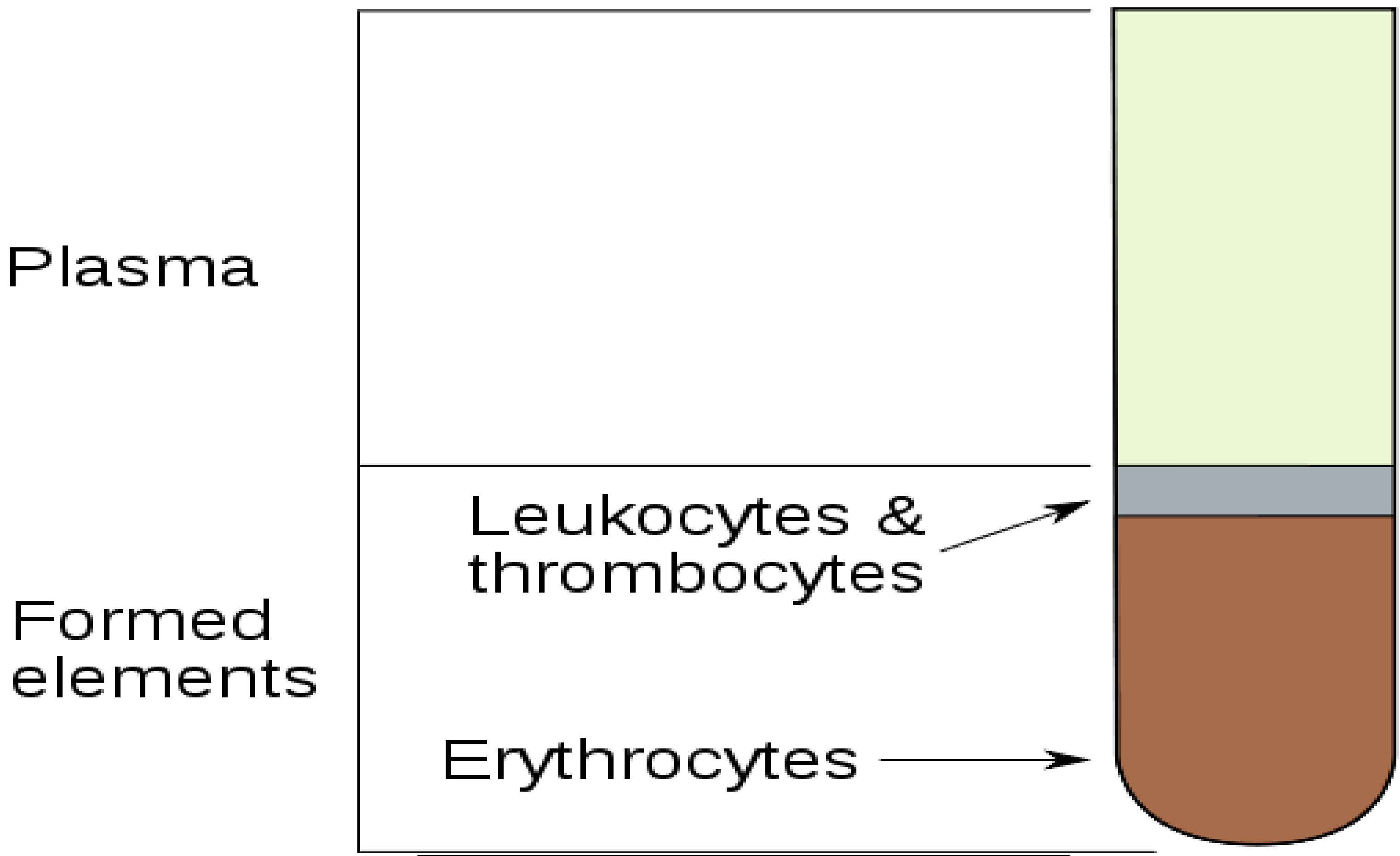

Figure H.1

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

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Hemoglobin **Hematocrit**

Are *not* indicators of an early iron deficiency

figure 9.1 page 323

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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

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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

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Mean Corpuscular Hemoglobin Concentration

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

Reference value: 320 – 360 g/L

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Mean Corpuscular Volume

Volume of the average RBC

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

Reference value: 80 – 100 fL

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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

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(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

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

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

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Ferritin Model

2 out of 3 tests should be abnormal

Overestimation of iron deficiency**

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

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MCV Model

2 out of 3 tests should be abnormal

Better than Ferritin model

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

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Ferritin Model **MCV Model**

Cannot distinguish iron-deficiency anemia from other causes of anemia

Because they include erythrocyte protroporphyrin as a variable

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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

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Folate Status

Assessment & Evaluation

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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

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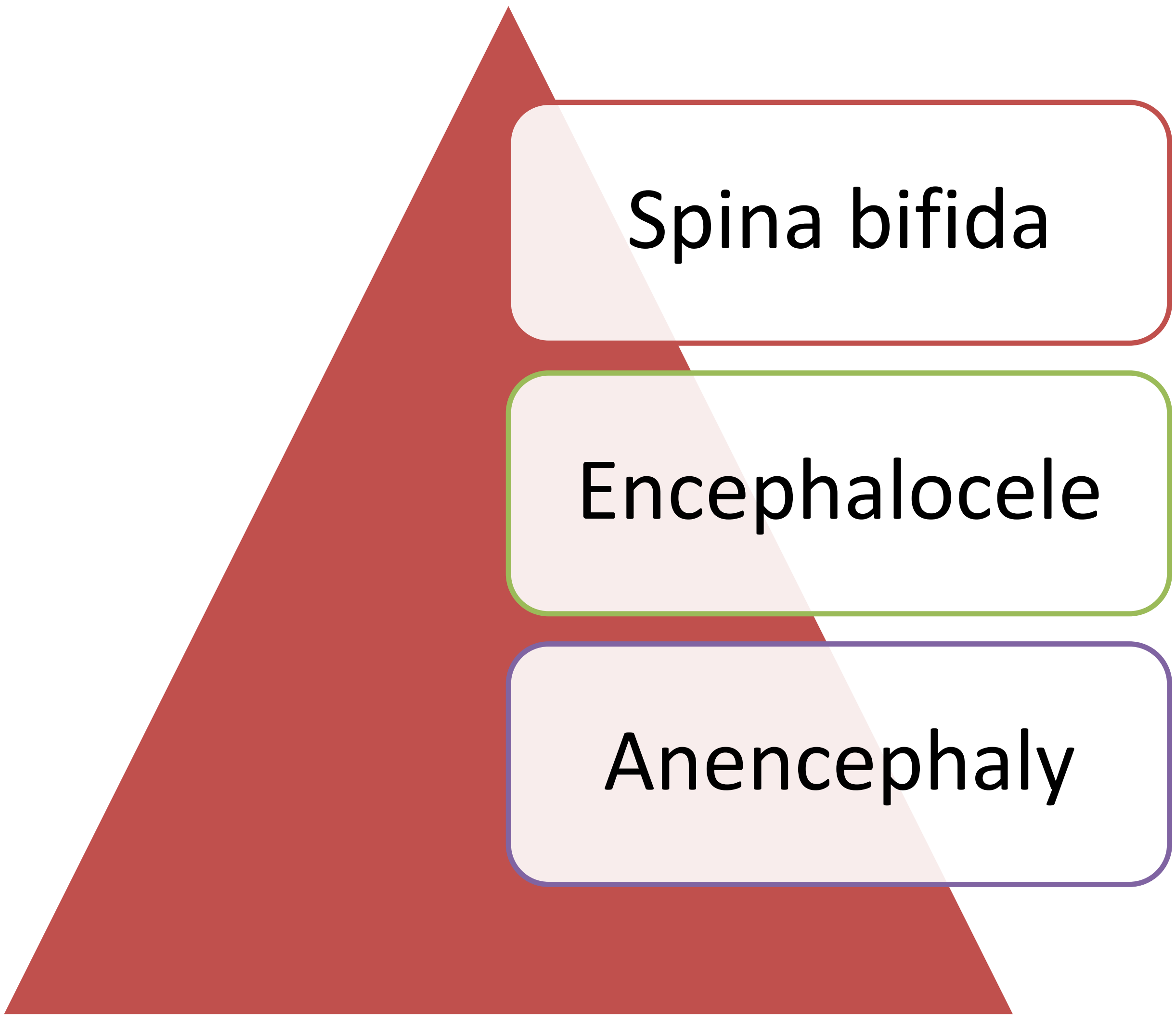
Clinical Features of folate deficiency



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Stages of folate deficiency

Positive homeostasis

Normal

Negative homeostasis

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Stages of folate deficiency

Normal

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

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Stages of folate deficiency

Positive homeostasis

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

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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 Liver folate < 1.2 µg/g	Change in RBC morphology ⬆MCV ⬇ HG

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Assessment of folate deficiency

Serum Folate

Erythrocyte folate

Deoxyuridine Suppression Test

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Serum Folate

< 3 ng/L

Fails to differentiate between the chronic and the transient folate deficiency

Alcohol

Smoking

Contraceptives

→ ↓ **SF**

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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**

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B12 STATUS

Assessment & Evaluation

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B 12

Deficiency Symptoms
Megaloblastic anemia
Nerve degradation

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

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Stages of B12 deficiency

Normal

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

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Stages of B 12 deficiency

Positive homeostasis

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

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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 ⬆MCV ⬇ HG

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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

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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)

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