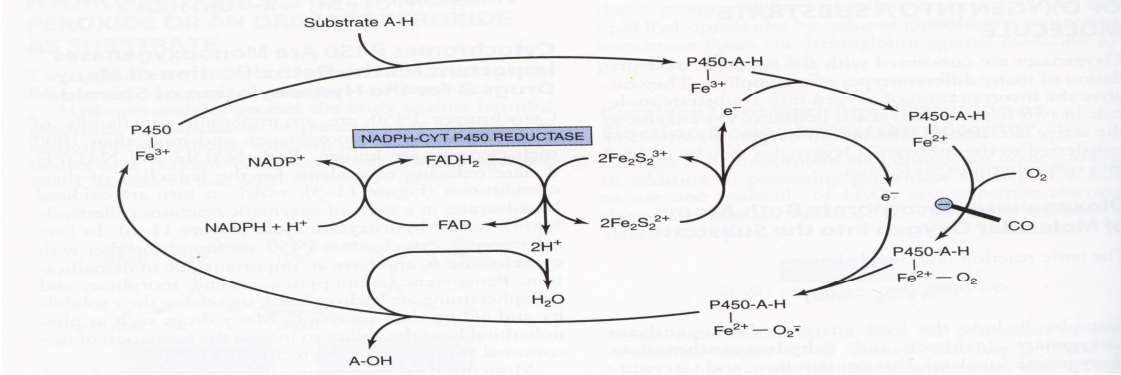

LABORATORY MANUAL

Fundamentals of Biomedical Science II

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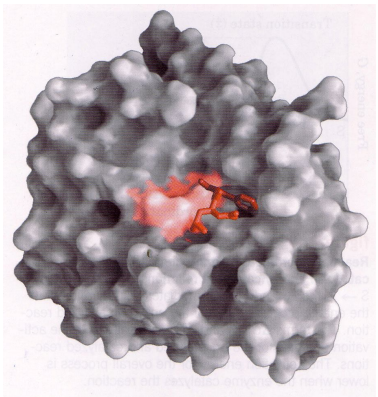

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PREFACE

In line with the idea to integrate basic medical science with clinical science, we introduce laboratory practical work based on clinical case extended with small group discussion. The purpose of this idea is to identify intellectual principles and concepts that can advance the integration of teaching and learning across the basic sciences and between the basic sciences and clinical medicine in medical education. This kind of structure of laboratory activity will not only enhance the psychomotor and knowledge competency of the students, but will also improve their behavior as well, as this program can endorse the students to build team work and have higher motivation to study medicine. This book is design as the manual for the students, which will guide them in doing their laboratory practical work and assist them to understand better the theory they learn during the lecture. Structurally this manual consists of general objective of the laboratory activity, patient's case presentation, topics to be discussed, which are related to topics they learn during the class lecture, laboratory methods, short review of the clinical case and some assignments.

Designed to support the understanding of topics delivered in Fundamentals of Biomedical Science II, this laboratory manual presents cases, topics for discussions and laboratory experiments related to redox system, thermodynamic, enzyme activity, energy metabolism and balance. Three cases, i.e., aspirin intoxication, duodenic ulcer, and metabolic syndrome, are designed as trigger for students to discuss several topics in basic medical science and as entry point to do some laboratory works, such as determining malondialdehyde production from fatty acid oxidation, methemoglobin assessment, observing factors that influence ptyaline's activity, assessment of AST activity, blood glucose determination, and nutritional status assessment.

Without support, encouragement and participation of many people, this book would never been accomplished. Our personal and very deep appreciation and acknowledgement goes to all of the contributors for accepting the challenge of preparing the contents, for sharing their ideas and recommendations to construct this manual, for accepting so readily suggestions to modify their contributions, and for their

understanding and cooperating throughout the period of the preparation. To each we extend our sincerest thanks for a job well done.

In any project, somebody must accept the responsibility for the final product. The decisions concerning the selection of topics and format, reviewing the drafts, and responsibility for the final checking of the manual were entirely ours. We welcome comments, criticisms, and suggestions from the students, faculty, colleagues, and professionals who read this manual. It is our hope that this work will be of a value to those searching on the exciting experience of learning biochemistry where the basic of medical science begins and is expanding so rapidly.

Editors

GENERAL INSTRUCTION

Before starting any experiment the student should read the instructions carefully, paying attention to all details, and should be quite certain about what he/she is trying to do. Be prepared for the discussion, read a lot about the discussion topic. During the discussion, do not hesitate to ask or to express opinion. Try to think and speak logically. Try to listen to your friend's opinion. The result of the experiment and discussion should be written and handed over to the tutor on the next meeting.

LABORATORY RULES

1. Do not replace any solution in a reagent bottle; take only the minimum amount required.
2. Replace the stopper immediately a solution has been taken from a bottle and take care not to mix stopper.
3. Do not remove bottles of special reagents from side shelves to your own benches.
4. Never mouth-pipette a corrosive fluid, always use pipette filler.
5. Cleanliness is essential in all biochemical work. Make sure that your glassware is clean and dry.
6. When you have finished work, leave your bench clean and dry, just as you expect to find it. See that all waste material is put in the wastebasket provided and not into the drains. Strong acids or bases should not be poured down the drain, unless accompanied by a large volume of water.
7. Be careful with the use of centrifuge or spectrophotometer equipment; do not use them before you know exactly how to operate them.
8. Inspect your the content of your bench every time before and after you use them. Any damage or loss must be reported immediately.
9. The person responsible for it must exchange damage or loss of the equipment in a short time.

CHAPTER I. REDOX SYSTEM AND THERMODYNAMIC

Living cells can interconvert different forms of energy and also may exchange energy with their environments. Therefore it is necessary to review the principles of thermodynamics, which regulate this type of reactions. Knowledge of this principle will facilitate a perception of how energy-producing and energy-utilizing metabolic reactions are permitted to occur within the same cell and how an organism is able to accomplish various work functions.

Bioenergetics, or biochemical thermodynamic, is the study of the energy changes related to biochemical reactions. Biologic systems are essentially isothermic and use chemical energy to power living processes. How human being obtains suitable fuel from food to provide this energy is basic to the understanding of normal nutrition and metabolism. The first law of thermodynamics states that the total energy of a system, including its surroundings, remains constant. It implies that within the total system, energy is neither lost nor increased during any change. However, energy may be transferred from one part of the system to another or may be transformed into another form of energy. In living systems, chemical energy may be transformed into heat, electrical, or mechanical energy. While the second law of thermodynamics states that the total entropy of a system must increase if a process is to occur spontaneously. Entropy is the extent of disorder or randomness of the system and becomes maximal as equilibrium is approach.

Chemically, oxidation is defined as the removal of electrons and reduction as the gain of electrons. Thus, oxidation is always accompanied by reduction of an electron acceptor. This principle of oxidation-reduction applies equally to biochemical systems and is an important concept underlying understanding of the nature of biologic oxidation. Note that many biological oxidations can take place without the participation of molecular oxygen, eg, dehydrogenations. The human life is absolutely dependent upon a supply of oxygen for respiration, the process by which cells derive energy in the form of ATP from the controlled reaction of hydrogen with oxygen to form water. In addition, molecular oxygen is incorporated into a variety of substrates by enzymes known as

oxygenases; many drugs, pollutants, and chemical carcinogens (xenobiotics) are metabolized by enzymes of this class, known as the cytochrom P450 system. Administration of oxygen can be lifesaving in the treatment of patients with respiratory or circulatory failure.

The following case presents a clinical problem where bioenergetics disturbance resulted from chemical intervention on a living organism, in this case is human.

The problem

This is a case of an eighteen month old boy who was admitted to the hospital with a Chief complaint: Cold, vomiting, and fever.

History of Present Illness :

According to the mother, the child had been well until three days prior to admission at which time the child developed a nonproductive cough, nasal congestion and a low grade fever. Because of these symptoms the mother began to treat the child with aspirin. Over the ensuing days the cold (congestion and cough) became no worse; however, the child developed vomiting and abdominal pain. The mother observed bluish color around the mouth and fingers. On the day of admission the child was quite listless and indeed was very difficult to arouse.

Past Medical History: For any significant major medical illness was unremarkable. Immunizations were current. Development was appropriate for his chronological age. Also, the child had been on no other medications prior to this illness.

Physical Examination: Temp., 38.5 ^{o}C; Pulse, 136/min; RR, 82/min and slightly labored; BP,90/60 mm Hg.

The child was very difficult to arouse even with noxious (painful) stimulation. Other results from physical examination were normal except slight bluish was found in extremities.

Laboratory examination revealed that Hb was 11.5 gm/dl (normal 11-13); WBC : 8,000/mm^3 (normal 5,000-11,000); Normal Diff. count, and MetHb. **: 7%** (normal < 2%)

Course :

Following admission it was suspected that the child represented a case of salicylate intoxication. The child was subsequently put on intravenous fluids with glucose. Intravenous bicarbonate was also administered while monitoring urine pH until its pH reach at least 7.

Additionally, the child underwent gastric lavage. Following this the child was given activated charcoal by a nasogastric tube. Also, the child underwent a lumbar puncture with spinal fluid analysis-normal. Over the ensuing hours the child's labored respiration gradually abated and the child became more alert. Initial salicylate level was 90 mg per 100 ml of blood.

Topics for discussion

1. Principle of thermodynamic
2. Principle of redox reaction
3. Redox system in living organism
4. Antioxidant

Overview of salicylate poisoning

Salicylates (aspirin, methyl salicylate, etc) are found in a variety of over-the-counter and prescription medications. Salicylates uncouple cellular oxidative phosphorylation, resulting in anaerobic metabolism and excessive production of lactic acid and heat, and they also interfere with several Krebs cycle enzymes. A single ingestion of more than 200 mg/kg of salicylate is likely to produce significant acute intoxication. Poisoning may also occur as a result of chronic excessive dosing over several days. Although the half-life of salicylate is 2-3 hours after small doses, it may increase to 20 hours with intoxication.

Acute ingestion often causes nausea and vomiting, occasionally with gastritis. Mild to moderate intoxication is characterized by hyperpnea (deep and rapid breathing),

tachycardia, tinnitus, and elevated anion gap metabolic acidosis. Serious intoxication may result in agitation, confusion, coma, seizures, cardiovascular collapses, pulmonary edema, hyperthermia, and death. The prothrombin time is often elevated owing to salicylate-induced hypoprothrombinemia.

Diagnosis is suspected in any patient with metabolic acidosis and is confirm by measuring the serum salicylate level. Patients with levels greater than 100 mg/dL (1000 mg/L) after an acute overdose are more likely to have severe poisoning. On the other hand, patients with chronic intoxication may suffer severe symptoms with levels of only 60-70 mg/L. The arterial blood gas typically reveals a respiratory alkalosis with an underlying metabolic acidosis.

Emergency and Supportive Measure: Maintain a clear airway, and assist ventilation if necessary. Treat coma, hyperthermia, hypotension, and seizures as described at the beginning of this chapter. Treat metabolic acidosis with intravenous sodium bicarbonate. After acute suicidal or accidental ingestion of more than 150-200 mg/kg salicylate, empty the stomach by emesis or gastric lavage and administer activated charcoal. Extra doses of activated charcoal may be needed in patients who ingest more than 10 g of aspirin.

Specific Treatment: Alkalinization of the urine enhances renal salicylate excretion by trapping the salicylate anion. Add 100 meq (two ampules) of sodium bicarbonate to 1 L of 5% dextrose in D5 0.2% saline, and infuse this solution intravenously at a rate of about 150-2—mL/h. Unless the patient is oliguric, add 20-30 meq of potassium to each liter of intravenous fluid. Hemodialysis may be lifesaving and is indicate for patients with severe metabolic acidosis, markedly altered mental status , or significantly elevated salicylate levels (eg, > 100-1200 mg/dL [1000-1200 mg/L] after acute overdose or > 60-70 mg/dL [600-700 mg/L] with chronic intoxication.

Blood methemoglobin assay

Methemoglobin is a form of hemoglobin in which the iron is oxidized to produce the iron (III) state. A tendency for methemoglobin to be present in excess of its normal level of about 1% may be due to a heredity defect of the globin chain or to exposure to oxidizing drugs or chemicals. Hemoglobin subunit containing this modified heme does not bind oxygen, but they change the oxygen-binding characteristics of the normal subunits in hybrid hemoglobin molecules containing some normal subunits and one or more modified subunits.

The ferrous iron of hemoglobin is susceptible to oxidation by superoxide and other oxidizing agents, forming methomoglobin, which cannot transport oxygen. Only a every small amount of methemoglobin is present in normal blood, as the red blood cell possesses an effective system (the NADH-cytochrome b_5 methemoglobinreductase system) for reducing heme F^{e3+} back to the F^{e2+} state. This system consists of NADH (generated by glycolysis), a flavoprotein named cytochrome b_5 reductase (also known as methemoglobin reductase), and cytochrome b_5. The F^{e3+} of methemoglobin is reduced back to the F^{e2+} state by the action of reduced cytochrome b_5:

$$\text{Hb-F}^{e3+} + \text{Cyt b}_{5\ red} \rightarrow \text{Hb-F}^{e2+} + \text{Cyt}_{\ b5\ ox}$$

Reduced cytochrome b_5 is then regenerated by the action of cytochrome b_5 reductase:

$$\text{Cyt b}_{5\ ox} + \text{NADH} \rightarrow \text{Cyt b}_{5\ red} + \text{NAD}$$

Methemoglobinemia can be classified as either inherited or acquired by ingestion of certain drugs and chemicals. Neither type occurs frequently, but physicians must be aware of them. The inherited form is usually due to deficient activity of methemoglobin reductase, transmitted in an autosomal recessive manner. In Hb M, mutation changes the amino acid residue to which heme is attached, thus altering its affinity for oxygen and favoring its oxidation. Ingestion of certain drugs (eg, sulfonamides) or chemicals (eg, aniline) can cause acquired methemoglobinemia. Cyanosis (bluish discoloration of

the skin and mucous membranes due to increased amounts of deoxygenated hemoglobin in arterial blood, or in this case due to increased amounts of methemoglobins) is usually the presenting sign in both types and is evident when over 10% of hemoglobin is in the "met" form. Diagnosis is made by spectroscopic analysis of blood, which reveals the characteristic absorption spectrum of methemoglobin. Additionally, a sample of blood containing methemoglobin cannot be fully reoxygenated by fusing oxygen through it, whereas normal deoxygenated blood can. Electrophoresis can be used to confirm the presence of an abnormal hemoglobin. Ingestion of methylene blue or ascorbic acid (reducing agents) is used to treat mild methemoglobinemia due to enzyme deficiency. Acute massive methemoglobinemia (due to ingestion of chemicals) should be treated by intravenous injection of methyl blue.

Principle of the experiment:

Methemoglobin can be determined spectrophotometrically at wavelength 630 nm after adding cyanide in the blood. The Cyanmethemoglobin will not absorb the spectrum at 630 nm. The difference in absorbance determines the methemoglobin. The addition of Potassium fericyanide will change all hemoglobin into methemoglobin. The absorbance before and after the addition of cyanide will determine the methemoglobin's concentration.

Objective:

Determining blood methemoglobin concentration.

Sample:

Fresh blood with anticoagulant heparin, EDTA or ACD (Acid Citrate Dextrose).

Materials:

1. $K_3Fe(CN)_6$
   Dissolve 2 gr $K_3Fe(CN)_6$ in 10 ml aquadest. Store in dark bottle at 4 ^oC.
2. KCN solution (poison).

Dissolve 500 mg KCN in 10 ml aquadest. Give label "POISON".

3. Buffer K. Phosphat 0.15 mol/l. pH 6.6 (20^oC).
   Dissolve 17.1 gr $K_2HPO_4.3H_2O$ (13,2 gr anhydrid) and 10.2 gr KH_2PO_4 separately in 500 ml aquadest.
   Mix in 9/16 v/v composition to get pH 6.6, store at 4^oC. Do not use if the solution become cloudy. This solution will be expired in 3 months.

Method:

1. Add 0.1 ml fresh blood into a tube containing 3.9 ml aquadest. Mix and shake the tube.
2. Add 4 ml K-phosphate buffer, mix and shake thoroughly.
3. Prepare blank cuvette with 1.5 ml buffer phosphate + 1.5 ml aquadest. Give label C_1.
4. Take each 3 ml hemolisate (from point 2), put into 2 cuvettes, and label with C_2 and C_3.
5. Add 0,1 ml $K_3Fe(CN)_6$ to C_3. Close with parafilm, mix and shake by turning it up and down three times. Let the cuvette in room temperature at least 2 minutes before read the absorbance
6. Read the absorbance at 630 nm from cuvette C_2 and C_3 against blank C_1.

Result:

$C_2 \longrightarrow A_{2a}$

$C_3 \longrightarrow A_{3a}$

7. Add 0.1 ml KCN into cuvette C_1, C_2 and C_3, close with parafilm and mix by turning it up and down three times. Let it 5 minutes before the absorbance is read.
8. Read again the absorbance at 630 nm from C_2 and C_3 against blank C_1.

Result:

$C_2 \longrightarrow A_{2b}$

$C_3 \longrightarrow A_{3b}$

9. Calculation:

$$\text{Methemoglobin (percent from total Hb)} = 100 \text{ X } \frac{A_{2a} - A_{2b}}{A_{3a} - A_{3b}}$$

Questions:

1. Explain factors that influence enzyme system which maintain blood methemoglobin concentration!
2. Explain the effect of methemoglobinemia on blood biochemistry!
3. Explain metabolic pathway which provides enzyme system to maintain methemoglobin concentration?

CHAPTER II. ENZYME ACTIVITY

Introduction:

Natural or prepared foods consumed by the average human adult are very complex mixture, although the majority of the components of a typical human diet can be divided as protein, carbohydrate, fat, vitamins, electrolytes and trace minerals. Ingested foods can be absorbed in an unchanged form, e.g. glucose, or must first be hydrolyzed by extensive enzymatic degradation as for muscle proteins. Enzymatic digestive processes are by now well understood, but the detailed mechanisms of these enzymatic reactions, the molecular mechanism, and the clinical implications have only been partially elucidated.

Enzymes are proteins that function in the acceleration of chemical reactions in biological systems. Many reactions required for the living cell would not proceed fast enough at the pH and temperature of the body without enzymes. The rationale of measuring plasma enzyme activities is based on the premise that changes in activities reflect changes that have occurred in a specific tissue or organ. Plasma enzymes are of two types: (1) one type is present in the highest concentration, is specific to plasma, and has a functional role in plasma; and (2) the second is normally present at a very low level and plays no functional role in the plasma. In the diagnosis of specific organ involvement in a disease process it would be ideal if enzymes unique to each organ could be identified.

The problem:

Mr. Dudi Naludi, a 54 years old male, executive in a real estate management firm was hospitalized with

Chief Complaint:

Vomiting, pain at **upper left abdominal region**

Past History:

For many years Mr. Naludi had experienced periodic "gnawing" epigastric pain that tended to appear about 2 hours after a meal and was usually promptly, though temporally, relieved by any one of a number of antacid preparations. On two occasions, an upper gastrointestinal series had revealed a deformed duodenal bulb; on a third such examination, performed after an episode of tarry stools two years before being hospitalized, the radiologist detected an ulcer crater in the center of the bulb.

Mr. Naludi could not identify any factors responsible for his symptomatic recurrences, although he admitted that he was constantly under pressure from large business responsibilities in a very competitive field.

Despite strong advice from several doctors, Mr. Naludi had been unable to reduce his daily consumption of about 40 cigarettes, and he continued to seek relaxation before his evening meal with two cans of beer.

About two months before his hospitalization he noted a change in the pattern of his symptoms; antacids no longer gave prompt or complete relief, he occasionally awoke at night with abdominal pain, and he had vomited large amounts of gastric contents on several occasions. He denied recent melena and the use of salicylates in any form, and ever got jaundice either.

Present history of illness:

Other than complaining on his pain, Mr. Naludi **experienced diarrhea**.

Physical examination:

BP: 140/90 mmHg; pulse 96 bpm and regular; no orthostatic change; respiration 18/min; temperature 37.5°C.

Eyes : No jaundice.

Lungs : Normal

Heart : Normal.

Abdomen : Protuberant and pain at the upper abdomen. Liver was unpalpable.

The remainder of the examination was negative.

Laboratory Tests:

	Patients	Normal
Blood analysis:		
WBC	Within normal limits	
Ht	45%	43-49%
Hb	13.5 g/dl	14.5-16.5 g/dl
RBC	$5x10^6/mm^3$	$4.7\text{-}6.1x10^6/mm^3$
Calcium, serum	10.1 mg/dl	9-11 mg/dl
Albumin	4.5 g/dl	3.8-4.8 g/dl
Bilirubin, total	1.0 mg/dl	0.2-1.2 mg/dl
SGOT (AST)	28 IU/L	22-37 IU/L
SGPT (ALT)	20 IU/L	3-36 IU/L
LDH	138 IU/L	100-190 IU/L
Amylase, serum	30 IU/L	23-85 IU/L

Urine analysis: Within normal limits.

Fecal analysis: Stool obtained on rectal examination was positive for occult blood.

Course:

Mr. Naludi's initial treatment consisted of continuous nasogastric suction and intravenous fluid replacement.

After several days of gastric decompression and ongoing ulcer therapy, he was begun on oral fluids, and gastric retention did not occur. Gastroscopy was performed, and confirmed the presence of **duodenal ulcer disease**. A gastric mucosal biopsy for ***Helicobacter pylori*** was obtained.

After a week of ulcer treatment, a gastric analysis was performed. The basal acid output was 5.3 mEq/h (Normal = 2.57 mEq/h), and the maximal acid output was 35 mEq/h (Normal, histamine stimulated = 22.6 mEq/h).

Measuring Aspartat Amino Transferase Activity.

Principle:

Aspartat Amino Transferase catalyses the transfer of Nitrogen from glutamate to oxaloacetate according to the following equation:

α - ketoglutarate + aspartate $\rightleftharpoons$ glutamate + oxaloacetate

For the quantitative AST determination the serum being tested is reacted in a buffered solution wish the ketoglutarate and aspartate. The resulting the presence of malate dehydrogenase (MDH) by NADH2 to malate, thus :

$$\text{Oxaloacetate + NADH2} \xrightleftharpoons{\text{MDH}} \text{malate + NAD}$$

The rate of $NADH_2$ consumption can be measured via the decrease in absorbance in the near UV region. It is directly proportional to the AST activity.

Materials:

1. Buffer – substrate solution.
2. Solvent

Procedure:

Add the solvent into substrate and mix well. After being dissolved the reagent can stand for 30 days at $2^0 – 8^0$ C.

Pipette into tube	Sample	Blank
Buffer – substrate solution	1.0 ml	1.0 ml
Serum	0.1 ml	
Aquadest		0.1 ml
Mix well at 37^0 C, after 1 (one) minute measure the absorbance at wavelength of 334, 340 or 365 nm each minute for 3 minutes, and note the average absorbance per minute [ΔA/minute]		

Calculation:

AST activity (IU/L): [ΔA/minute] x factor

Factor:

Wavelength (nm)	334	340	365
Factor	1780	1746	3235

Normal value:

	30^0 C	37^0 C
Male	6 - 25	8 – 37
Female	6 - 21	8 – 31

Topic of discussion

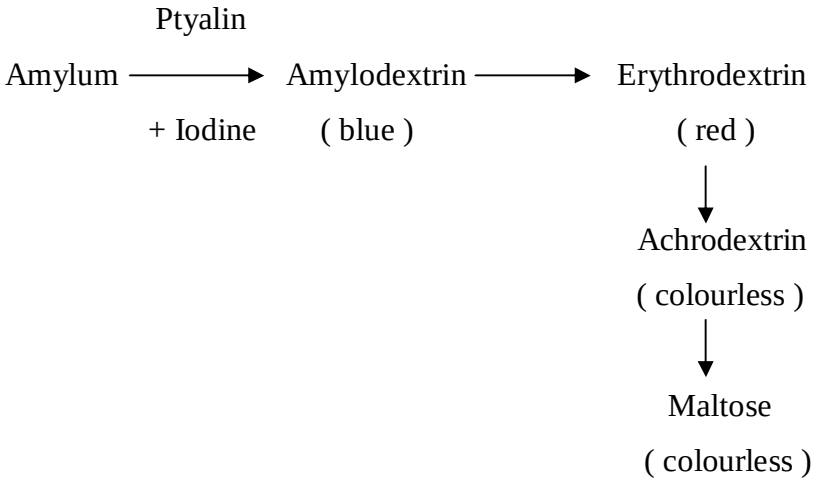
1. In what condition is AST activity increased in the blood?
2. Explain the use of enzyme for diagnostic of diseases.

Determining factors which influence amylase activity

Saliva is produced by the submaxillary, sublingual and parotid glands, as well as by the mucous membrane and buccal glands of the mouth, throat and œsophagus. Saliva contains about 99,5 percent of water. It also contains ptyalin (or salivary amylase), several proteins (of which mucin, a glycoprotein, is the most important) and a number of substances found in blood and urine (such as ammonia, amino acids, urea, uric acid, cholesterol, calcium, sodium, potassium, magnesium, phosphate, chloride and bicarbonate).

Principle:

Ptyalin (or salivary amylase) acts on starch, producing a series of ill-defined products: soluble starch, erythrodextrin, achrodextrin and maltose. The activity of salivary amylase is enhanced by the chloride ion. Salivary amylase acts at the 1,4-glucosidic linkages of amylase and amylopectin.



Materials :

1. Starch solution 1%
2. Sodium chloride solution 1%
3. Sodium chloride solution 0,1%
4. Chloride 1 N
5. Iodine solution
6. Saliva

Collection of saliva:

After rinsing your mouth thoroughly with water, chew on a small piece of paraffin wax. This will stimulate the flow of saliva. Transfer the accumulated saliva to a small beaker, and use for the following experiment.

Procedure:

1. To 3 ml of a 1% starch solution in a test tube, add 1 ml of sodium chloride 0.1 % and 1 ml of saliva, add 3 drops of 0.01 M iodine, and place the tube in a water bath at 37 ° C for 10 minutes. Note the color changes.
2. To 3 ml of 1% starch solution in a test tube, add 1 ml of sodium chloride 1% and 1 ml of saliva, add 3 drops of 0.01 M iodine, and place the tube in water bath at 37 ° C for 10 minutes. Note the color changes.

3. To 3 ml of a 1% starch solution in a test tube, add 1 ml of chloride 1 N 1 ml of saliva, add 3 drops of 0,01 M iodine, and place the tube in water bath at 37 ° C for 10 minutes. Note the color changes.
4. To 3 ml of a 1% starch solution in a test tube, add 1 ml aquadest and 1 ml of saliva, add 3 drops of 0.01 M iodine, and place the tube in water bath at 37 ° C for 10 minutes. Note the color changes.
5. To 3 ml of a 1% starch solution in a test tube, add 1 ml sodium chloride 1% and 1 ml saliva, add 3 drops of 0.01 M iodine, and place the tube in water bath at 75 ° C for 10 minutes. Note the color changes.
6. To 3 ml of a 1% starch solution in a test tube, add 1 ml sodium chloride 1% and 1 ml saliva, add 3 drops of 001 M iodine, and place the tube in ice for 10 minutes. Note the color changes.

Topic of discussion:

1. Mention factors that influence enzyme activity and explain the mechanism!
2. What is an enzyme activator?
3. Which of the above ions is the best activator of salivary amylase?
4. Devise a quantitative experiment to determine the effect of temperature on Ptyalin activity?
5. What is the optimum pH for Ptyalin action?
6. How do you explain the continued action of ptyalin in the stomach?
7. What are the degradation products obtained when ptyalin acts on starch?

CHAPTER III. ENERGY METABOLISM, BALANCE & CONSUMPTION

Living cells are composed of a complex, intricately regulated system of energy-producing and energy-utilizing chemical reactions called metabolism. Metabolism consists of two contrasting processes, catabolism and anabolism, which represent the sum of chemical changes that convert foodstuffs into usable forms of energy and into complex biological molecules. Catabolism involves degradation of ingested foodstuffs or stored fuels such as carbohydrate, lipid, and protein into either usable or storable forms of energy. The reactions generally result in conversion of large complex molecules to smaller molecules (ultimately CO_2 and H_2O), and in mammals often require consumption of O_2. Energy-utilizing reactions perform various necessary and, in many instances, tissue-specific, cellular functions, for example, nerve impulse conduction, muscle contraction, growth, and cell division. Catabolic reactions are generally exergonic with the released energy generally trapped in the formation of ATP. The oxidative reactions of catabolism also result in transfer of reducing equivalents of the coenzymes NAD^+ and $NADP^+$ to form NADH and NADPH and a proton (H^+). Anabolic pathways are involved in biosynthesis of large, complex molecules from smaller precursors and require expenditure of energy either in the form of ATP or using reducing equivalents stored in NADPH.

Changes in body energy content occur through changes in the balance between daily intake and energy expenditure. Energy intake is episodic, derived primarily from carbohydrate, proteins, and fats in foods consumed. Total daily energy expenditure can be divided into several components, i.e., thermic effect of activity (TEA) ~ 15 – 30 %, thermic effect of feeding (TEF) ~ 10 %, and resting metabolic rate ~ 60 – 75 %.

The understanding and assessment of energy requirements in humans have been enhanced by the advent of indirect calorimetry. In indirect calorimetry, the type and rate of substrate oxidation and energy are measured in vivo from gas exchange measurements. This method in combination with other measurement techniques permits investigation of numerous aspects of metabolism, heat production, energy requirements

of physical activity, and altered energy metabolism in injury and disease. The following case presents an example of imbalance energy consumption and expenditure, which results to a syndrome due to metabolism imbalance.

The problem

In a routine general check up, Mr. Obelix, a 41 year old GM from a finance firm was told by the physician to have metabolic syndrome. The following is part of the laboratory results from his check up,

Body weight	: 82 kg
Height	: 165 cm
Abdominal circumference	: 102 cm
Blood pressure	: 150/95 mmHg
Pulse	: 102 x/min.
Blood glucose	: 126 mg% fasting (N : 70 – 110 mg%)
	184 mg% 2 hrs pp (N : < 140 mg%)
Total cholesterol	: 240 mg% (N: 150 – 220 mg%)
HDL-cholesterol	: 28 mg% (N > 35 mg%)
LDL-cholesterol	: 170 mg% (N : 100 – 150 mg%)
Triglyceride	: 270 mg% (N : 150 – 250 mg%)

Due to his tight schedule of meetings he told the doctor that he can't control his diet because he always gets heavy and high calories meals during meetings, such as *nasi padang komplit*, many kind of pastry, with less vegetables, and he almost never does any exercise. Based on this situation, besides giving Mr. Obelix lipid lowering drug and oral anti diabetic, the physician put him in a diet program of 1800 kcal per day and Mr. Obelix should join Gymnastic program to improve his fitness.

Topics of discussion:

1. Energy metabolism
2. Energy balance
3. Nutritional status

Nutritional status assessment

Nutritional status assessment is essential, especially in children, for identifying either the undernourished or over nourished state and estimating the optimum energy intake to promote growth and well-being. In children with moderate-to-severe disabilities or many of the chronic diseases, nutritional assessment is complicated by interaction of the primary diseases process (i.e., muscle atrophy, contractures, chronic malabsorption), drug-nutrient interactions, and acute and/or chronic malnutrition.

Nutritional assessment has several components, including evaluation of dietary intake, growth status, body composition, energy expenditure, and laboratory data in the context of the medical history, diagnoses, and current therapy. The elements of complete nutritional assessment are based on the standard medical evaluation that includes a medical history, a physical examination, and laboratory assessment. Nutritional status is usually assessed by the primary physician, caregiver, physician's assistant, or nurse practitioner, though others (dieticians, speech therapists, occupational and physical therapists, social workers) may also participate in the assessment, depending upon the setting. Because nutritional assessment is multidisciplinary, cooperation of the interdisciplinary team is important. Dietary and biochemical assessment are important tools for evaluating the nutritional status. No single test provides an adequate measure of overall nutritional status; instead evaluation is based on variety of somewhat non specific indicators.

Determining the optimal approach to nutritional assessment in the clinical setting is difficult because non-nutritional factors alter many of the parameters used to determine nutritional status. For example, the serum albumin level can be affected by body fluid redistribution, sepsis, hepatic or renal disease, or the postoperative state. Furthermore, the long half-life of serum albumin makes this measurement insensitive to rapid changes in nutritional status. Urine and faecal nitrogen excretion and immune skin tests require up to 48 hours for results. Difficulty in obtaining complete 24-hour collections of urine and faeces poses another potential clinical limitation on assessment of nitrogen balance. While there is no single irrefutable measure of nutritional status, proficiency in

detecting malnutrition in its early stages is essential for effective treatment and prevention of adverse clinical outcomes, and interval assessments of nutritional status are required to evaluate the efficacy of any nutritional intervention. The ultimate assessment of nutritional status is a reliable determination of the balance (positive, negative or zero) between dietary intake and total loss from the body of a particular nutrient that is unchanged in metabolism (e.g., a mineral) or a basic element that is a marker for a nutrient (e.g., nitrogen). The diet and all excreta must be chemically analyzed. The patient's intake must be equilibrated on a standardized diet for varying times prior to complete collection of faecal, urinary, and any other losses from the body for 72 hours or longer. Appropriate losses in sweat, hair, and nails must be assumed for particular nutrients. In this chapter some of biochemical laboratory assessment will be practiced to determined nutritional status.

DETERMINATION OF TRIGLYCERIDES IN BLOOD WITH REFLOTRON®

Introduction

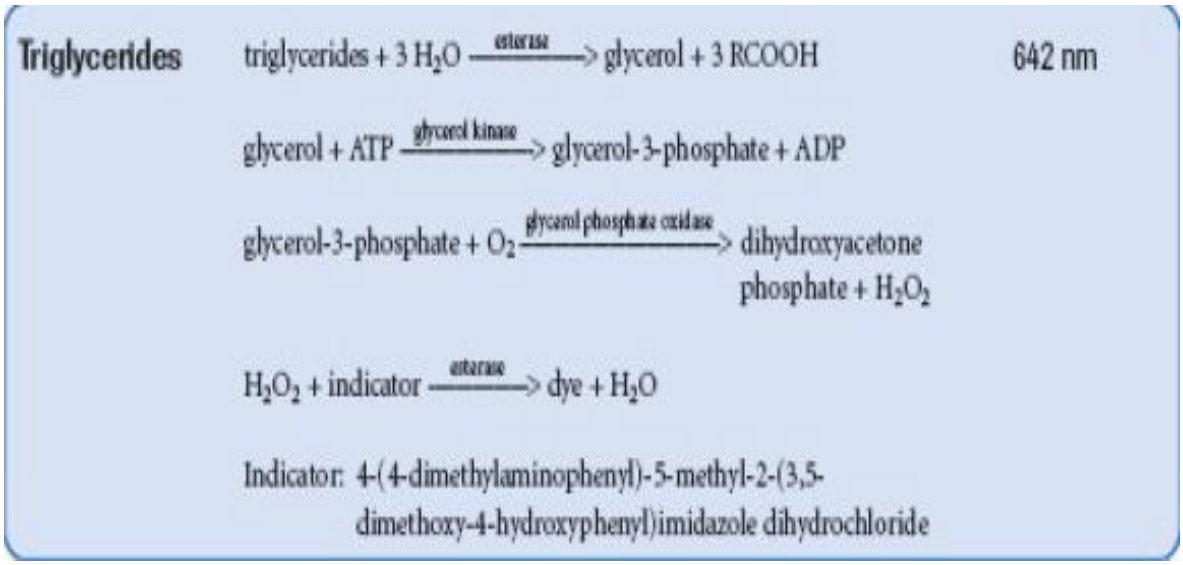
Triglycerides are esters of the tryhidric alcohol glycerol with 3 long-chain fatty acids. They are partly taken up with the diet, partly synthesized in the liver. Triglycerides are determined for early detection of a risk of atherosclerosis, for classification of hyperlipoproteinemia and for monitoring lipid-lowering diet therapy and pharmacotherapy.

Principle of Triglycerides Determination

Reflotron® is a compact reflectance photometer for fully automatic evaluation of Reflotron® Tests Strips. The instrument takes charge of all functions such as heating, automatic calibration, test execution and evaluation, and calculation of results.

After application to the strip test, the sample flow into the reaction zone, in the case of blood after separation of the erythrocytes from the plasma. The triglycerides are cleaved in an enzymatic reaction. Various reactions then lead to the formation of H_2O_2. This

oxidizes a redox indicator to a blue dye in a reaction catalyzed by the enzyme peroxidase:



At the temperature of 37^0C the dye formed is measured at 642 nm and the triglyceride concentration displayed after about 180 seconds in mg/dl or mmol/l.

Materials

1. Capillary Blood sample: 30 micro liters
2. Micro pipette
3. Reflotron® Triglycerides Reagent Strip (REF: 1 0745049)
4. Reflotron® Instrument
5. Blood lancet

Reagen Strip for Reflotron®; top and underside

Reflotron® Instrument

Procedure:

1. Prepare the Reflotron® triglycerides strip and unwrap the strip, taking care not to bend it
2. Swap the finger where you want to withdraw capillary blood using the alcohol swap
3. Withdraw capillary blood using blood lancet
4. Using the micro pipette, draw the sample material (about 30 micro liters) into the pipette (avoiding bubbles) and apply as a drop to the center of the red application zone (xx) – being careful not to touch the application zone with the pipette tip (see procedure in picture)
5. With the sliding cover or flap open, place the test strip on to the guide within 15 seconds and slide forward horizontally until it locks into place and close the flap
6. The instrument displays "TG" to confirm that it has correctly read in the test-specific magnetic code. The display shows the number of seconds left before the result is displayed. The triglyceride concentration is calculated automatically from the readings taken using a function and conversion factors that are entered in the instrument via the magnetic strip on the underside of each test strip.
7. The triglyceride concentration is displayed in mg/dl
8. Remove the used strip from the Reflotron® and dispose of it according your laboratory procedure

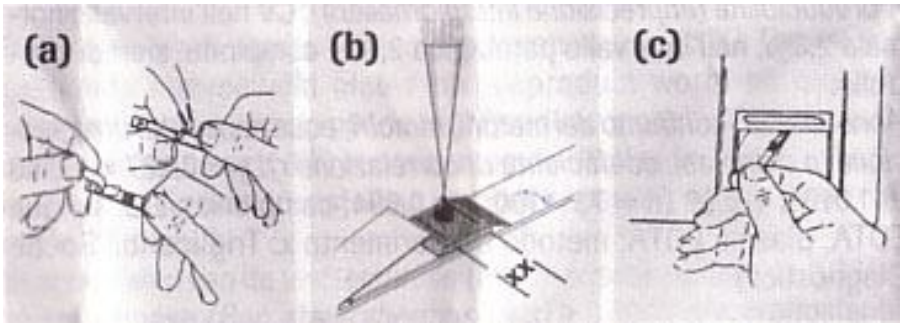
Measuring Range

The measuring ranges cover the entire clinically relevant concentration or activity range. $\leq$ 200 mg/dl or 2.30 mmol/l is normal.

References

1. Reflotron® Information Manual Procedure Booklet, Bio-Stat Diagnostic System, 2006
2. Reflotron® Triglycerides Reagent Strip Information Booklet
3. Harper
4. Devlin

Procedures in Pictures



NUTRITIONAL STATUS ASSESSMENT

Introduction

Nutritional status assessment evaluates a person's health from a nutrition perspective. Factors influence nutrition status include:

1. Historical information

Present nutrition status become evident with a review of a person's historical data:

a. health history: health factors that affect nutrition status
b. socio-economic history: personal, financial, environmental influences on food intake
c. drug history: medications and nutrition supplements
d. diet history: nutrient intake excesses or deficiencies

2. Anthropometric measurements

a. Height measurement
b. Weight measurement
c. Fat-fold measurement

3. Physical examination

Assessor can use a physical examination to search for signs of nutrient deficiency or toxicity. Such examination requires knowledge and skills.

4. Biochemical analysis

Biochemical analyses or laboratory tests help to determine what is happening inside the body. Common tests are based on analysis of blood and urine samples, which contain nutrients, enzymes, and metabolites that reflect nutrition status. The interpretation of biochemical data requires skill.

Principles and Procedure of Anthropometric Measurements

1. Height Measurement

The procedure for measuring a child who can stand erect and cooperate is the same as for an adult. The best way to measure standing height is with the person's back

against a flat wall to which a non-stretchable measuring tape or stick has been fixed. The person stands erect, without shoes, with heels together. The person's line of sight should be horizontal, with the heels, buttock, shoulders and head touching the wall. The assessor places a block, book, or other inflexible object on top of the head at right angle to the wall; carefully checks the height measurement; and records it immediately in either inches or centimeter. Such a practice prevents forgetting the correct measurement.

2. Weight Measurement

Valid weight measurements require scales that has been carefully maintained, calibrated and checked for accuracy at regular intervals. Beam balance and electronic scales are the most accurate types of scales. To measure infant's weight, assessors use special scales that allow infants to lie or sit. Weighing infants naked, without diapers, is standard procedure. Children who can stand are weighed in the same way as adult. To make repeated measures useful, standardized conditions are necessary. Each weighing should take place at the same time of day (preferably before breakfast), in the same amount of clothing (without shoes), after the person has voided, and on the same scale. Special scales and hospitals beds with built-in scales are available for weighing people who bedridden. Bathroom scales are inaccurate and inappropriate in a professional setting. As with all measurements, the assessor records the observed weight immediately in either pounds or kilograms.

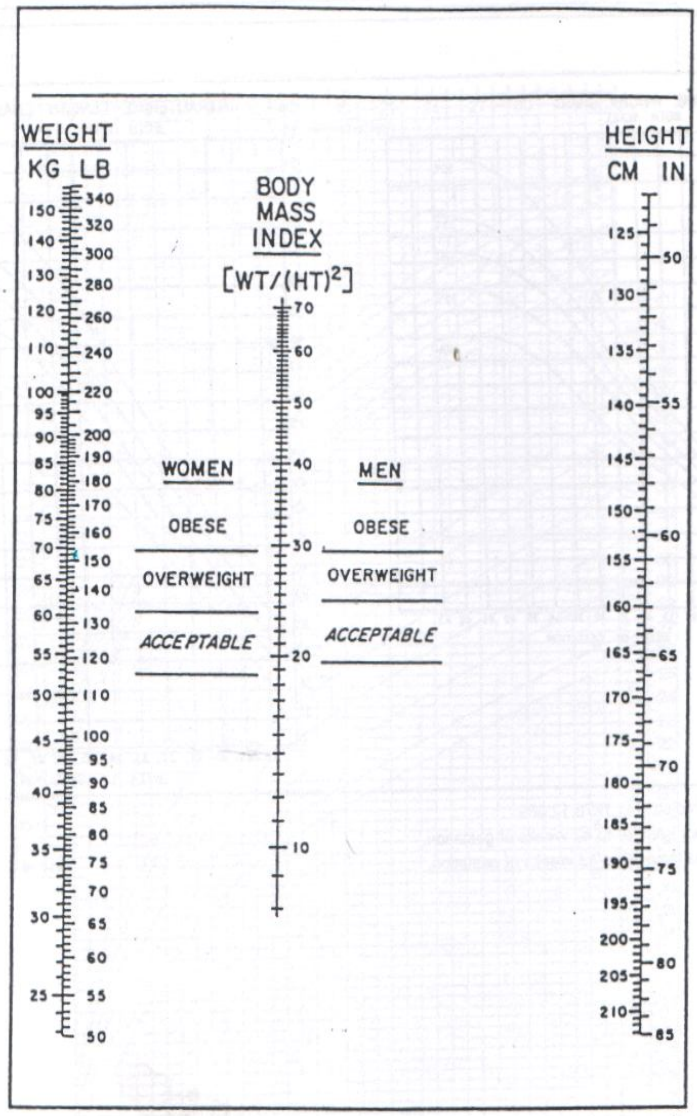
Body mass index measurement and waist circumference

Body Mass Index (BMI) can be calculated using any the following formula:

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{Height (m)}^2}$$

Or use nomograph below!

A-13-b
Nomograph for Estimating Body Mass Index (kg/m²)*



*The ratio of weight/height² emerges from varied epidemiologic studies as the most generally useful index of relative body mass in adults. This nomograph facilitates use of this relationship in clinical situations. While showing the range of weight given as desirable in life insurance studies, the scale expresses relative weight as a continuous variable. This method encourages use of clinical judgment in interpreting "overweight" and "underweight" and in accounting for muscular and skeletal contributions to measured mass. From G. A. Bray, 1978.

Figure: Nomograph for estimating Body Mass Index (BMI)

Waist circumference
Waist circumference is obtained by measuring the distance around the smallest area below the rib cage and above the umbilicus (belly button) with the use of a non-stretchable tape measure. Waist circumference measurements assess abdominal fat content.
Compare your result with classification below, and discuss in your group!!

Classification	**BMI (kg/m^2)**	**Risk of co-morbidities**	
		Waist Circumference	
		< 90 cm (men) **< 80 cm (women)**	**≥ 90 cm (men)** **≥ 80 cm (women)**
Underweight	< 18.5	Low(but increased risk of other clinical problems)	Average
Normal	18.5-22.9	Average	Increased
Overweight:	≥ 23		
At risk	23-24.9	Increased	Moderate
Obese I	25-29.9	Moderate	Severe
Obese II	≥ 30	Severe	Very severe

WHO-WPRO (2000)

3. Triceps Fat-fold Measurement

A. Find the midpoint of the arm:

1) Ask the subject to bend his or her arm at the elbow and lay the hand across the stomach. (if he or she is right-handed, measure the left arm and vice versa)
2) Feel the shoulder to locate the acromion process. The olecranon process is the tip of the elbow.
3) Place a measuring tape from the acromion process to the tip of the elbow. Divide this measurement by 2, and mark the midpoint of the arm with a pen.

B. Measure the Fat-fold:

1) Ask the subject to let his or her arm hang loosely to the side.
2) Grasp a fold of skin and subcutaneous fat between the thumb and forefinger slightly above the midpoint mark. Gently pull the skin away from the underlying muscle. (This step takes a lot of practice. If you want to be sure you don' have muscle as well as fat, ask the subject to contract and relax the muscle. You should be able to feel if you are pinching muscle.)
3) Place the calipers over the fat fold at the midpoint mark, and read the measurement to the nearest 1.0 milimeter in two to three seconds. (if using plactic calipers, align pressure lines, and read the measurement to the nearest 1.0 milimeters in two to three seconds)
4) Repeat steps 2 and 3 twice more. Add the three readings, and then divide by 3 to find the average.

(Please see the step on the below picture).

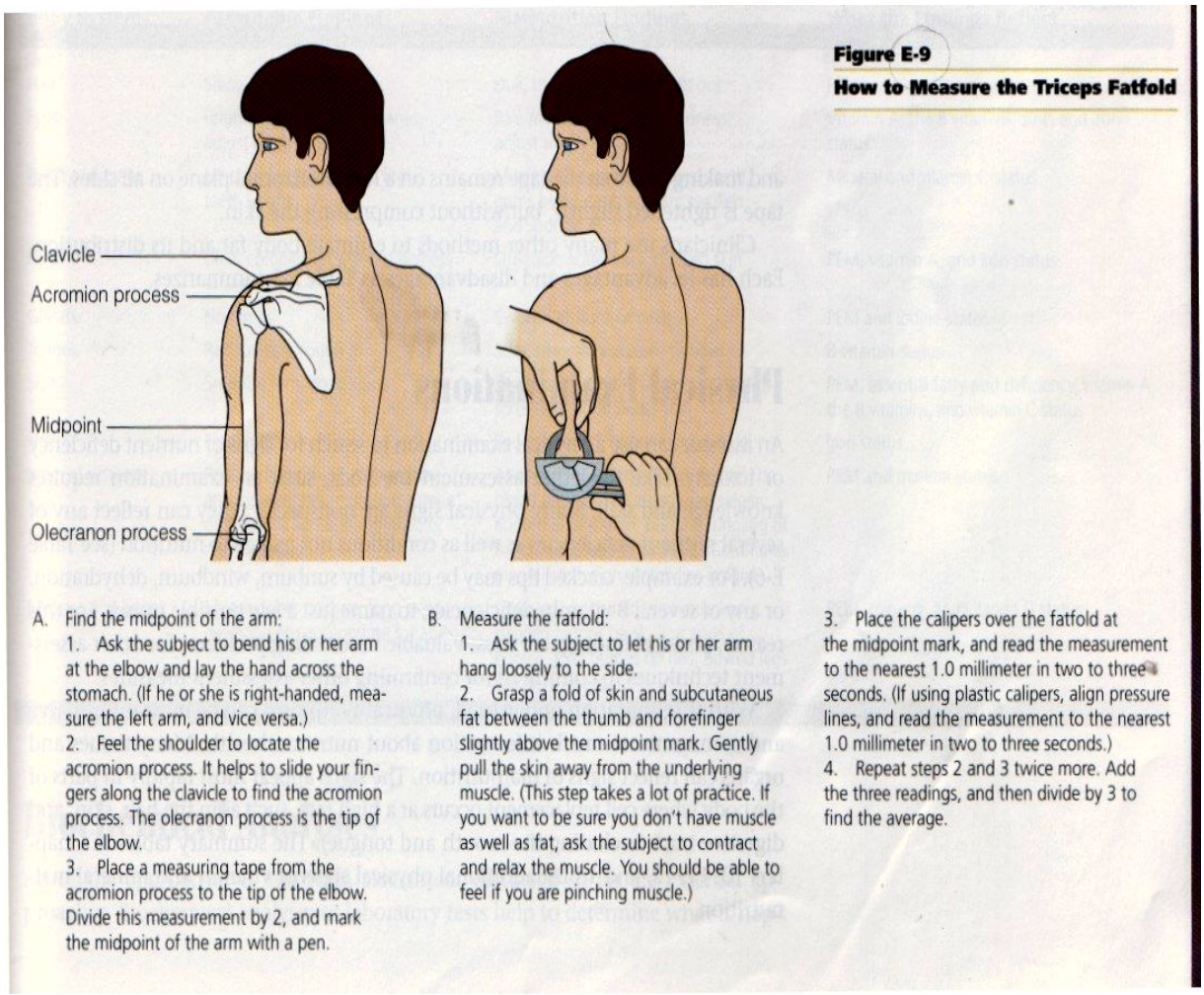


Figure E-9
How to Measure the Triceps Fatfold

A. Find the midpoint of the arm:
1. Ask the subject to bend his or her arm at the elbow and lay the hand across the stomach. (If he or she is right-handed, measure the left arm, and vice versa.)
2. Feel the shoulder to locate the acromion process. It helps to slide your fingers along the clavicle to find the acromion process. The olecranon process is the tip of the elbow.
3. Place a measuring tape from the acromion process to the tip of the elbow. Divide this measurement by 2, and mark the midpoint of the arm with a pen.

B. Measure the fatfold:
1. Ask the subject to let his or her arm hang loosely to the side.
2. Grasp a fold of skin and subcutaneous fat between the thumb and forefinger slightly above the midpoint mark. Gently pull the skin away from the underlying muscle. (This step takes a lot of practice. If you want to be sure you don't have muscle as well as fat, ask the subject to contract and relax the muscle. You should be able to feel if you are pinching muscle.)
3. Place the calipers over the fatfold at the midpoint mark, and read the measurement to the nearest 1.0 millimeter in two to three seconds. (If using plastic calipers, align pressure lines, and read the measurement to the nearest 1.0 millimeter in two to three seconds.)
4. Repeat steps 2 and 3 twice more. Add the three readings, and then divide by 3 to find the average.

Procedures in figure (see above)

Table E-4

Triceps Fatfold Percentiles (Millimeters)

	Male					Female				
Age	***5th***	***25th***	***50th***	***75th***	***95th***	***5th***	***25th***	***50th***	***75th***	***95th***
1–1.9	6	8	10	12	16	6	8	10	12	16
2–2.9	6	8	10	12	15	6	9	10	12	16
3–3.9	6	8	10	11	15	7	9	11	12	15
4–4.9	6	8	9	11	14	7	8	10	12	16
5–5.9	6	8	9	11	15	6	8	10	12	18
6–6.9	5	7	8	10	16	6	8	10	12	16
7–7.9	5	7	9	12	17	6	9	11	13	18
8–8.9	5	7	8	10	16	6	9	12	15	24
9–9.9	6	7	10	13	18	8	10	13	16	22
10–10.9	6	8	10	14	21	7	10	12	17	27
11–11.9	6	8	11	16	24	7	10	13	18	28
12–12.9	6	8	11	14	28	8	11	14	18	27
13–13.9	5	7	10	14	26	8	12	15	21	30
14–14.9	4	7	9	14	24	9	13	16	21	28
15–15.9	4	6	8	11	24	8	12	17	21	32
16–16.9	4	6	8	12	22	10	15	18	22	31
17–17.9	5	6	8	12	19	10	13	19	24	37
18–18.9	4	6	9	13	24	10	15	18	22	30
19–24.9	4	7	10	15	22	10	14	18	24	34
25–34.9	5	8	12	16	24	10	16	21	27	37
35–44.9	5	8	12	16	23	12	18	23	29	38
45–54.9	6	8	12	15	25	12	20	25	30	40
55–64.9	5	8	11	14	22	12	20	25	31	38
65–74.9	4	8	11	15	22	12	18	24	29	36

NOTE: If measurements fall between the percentiles shown here, the percentile can be estimated from the information in this table. For example, a measurement of 7 millimeters for a 27-year-old male would be about the 20th percentile.

Source: Adapted from A. R. Frisancho, New norms of upper limb fat and muscle areas for assessment of nutritional status. *American Journal of Clinical Nutrition* 34 (1981): 2540–2545.

Table: Triceps Fatfold Percentiles (Milimeters)

Table E-6

Physical Findings Used in Nutrition Assessments

Body System	Acceptable Findings	Malnutrition Findings	What the Findings Reflect
Hair	Shiny, firm in the scalp	Dull, brittle, dry, loose; falls out	PEM
Eyes	Bright, clear pink membranes; adjust easily to light	Pale membranes; spots; redness; adjust slowly to darkness	Vitamin A, the B vitamins, zinc, and iron status
Teeth and gums	No pain or caries, gums firm, teeth bright	Missing, discolored, decayed teeth; gums bleed easily and are swollen and spongy	Mineral and vitamin C status
Face	Clear complexion without dryness or scaliness	Off-color, scaly, flaky, cracked skin	PEM, vitamin A, and iron status
Glands	No lumps	Swollen at front of neck	PEM and iodine status
Tongue	Red, bumpy, rough	Sore, smooth, purplish, swollen	B vitamin status
Skin	Smooth, firm, good color	Dry, rough, spotty; "sandpaper" feel or sores; lack of fat under skin	PEM, essential fatty acid deficiency, vitamin A, the B vitamins, and vitamin C status
Nails	Firm, pink	Spoon-shaped, brittle, ridged, pale	Iron status
Internal systems	Regular heart rhythm, heart rate, and blood pressure; no impairment of digestive function, reflexes, or mental status	Abnormal heart rate, heart rhythm, or blood pressure; enlarged liver, spleen; abnormal digestion; burning, tingling of hands, feet; loss of balance, coordination; mental confusion, irritability, fatigue	PEM and mineral status
Muscles and bones	Muscle tone; posture, long bone development appropriate for age	"Wasted" appearance of muscles; swollen bumps on skull or ends of bones; small bumps on ribs; bowed legs or knock-knees	PEM, mineral, and vitamin D status

Table: Physical Findings Used in Nutrition Assessments

Reference:

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13. Shils ME, Olson JA, Shike M, Catharine Ross A. 1999. Modern Nutrition in Health and Disease: Body Mass Index. 9th edition. Williams & Wilkins. Appendix A-75

Schedule for Fundamentals of Biomedical Science-II - Laboratory activity

Case 1:
Aspirin intoxication

Laboratory activities:
Methemoglobin determination

Topics for discussion:
1. Principle of thermodynamic
2. Principle of redox reaction
3. Redox system in living organism
4. Antioxidant

Case 2:
Duodenic ulcer

Laboratory activities:
1. Plasma AST assay
2. Determining factors that influence ptyalin activity

Topic of discussion:
1. Explain factors that influence enzyme activity!
2. What is an enzyme activator?
3. How do you explain the continued action of ptyalin in the stomach?
4. In what condition is AST activity increased in the blood ?
5. Explain the use of enzyme for diagnostic of diseases.

Case 3:
Metabolic syndrome

Laboratory activities:
1. Blood triglyceride assay
2. Body mass index determination

Topics of discussion:
1. Energy metabolism
2. Energy balance
3. Nutritional status

Session	Case	Topic of Discussion	Laboratory Activities
1	Aspirin intoxication	Thermodynamic law and bioenergetics	Methemoglobin determination
2	Aspirin intoxication	Redox system in living organism and antioxidants	Lipid peroxidation determination (TBARS)
3	Duodenal ulcer	Factors that influence enzyme activity & mechanism of enzyme action	Ptyalin enzyme activity
4	Duodenal ulcer	Clinical aspect of enzyme	Plasma AST assay
5	Metabolic syndrome	Energy metabolism & balance	Blood glucose assay
6	Metabolic syndrome	Nutritional assessment	Body mass index assessment
7	-	Review all materials	-
8	SOCA	-	-
9	SOCA	-	-

Tutors :