



BCH 447



Lipase Assay

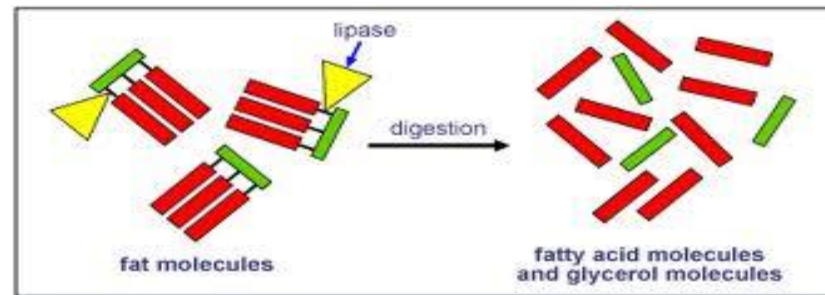
(Using Turbidimetric Method)

Objective

- To determine Lipase activity.

Lipase

- It is an enzyme that breaks down dietary fats into smaller molecules, fatty acids and glycerol.

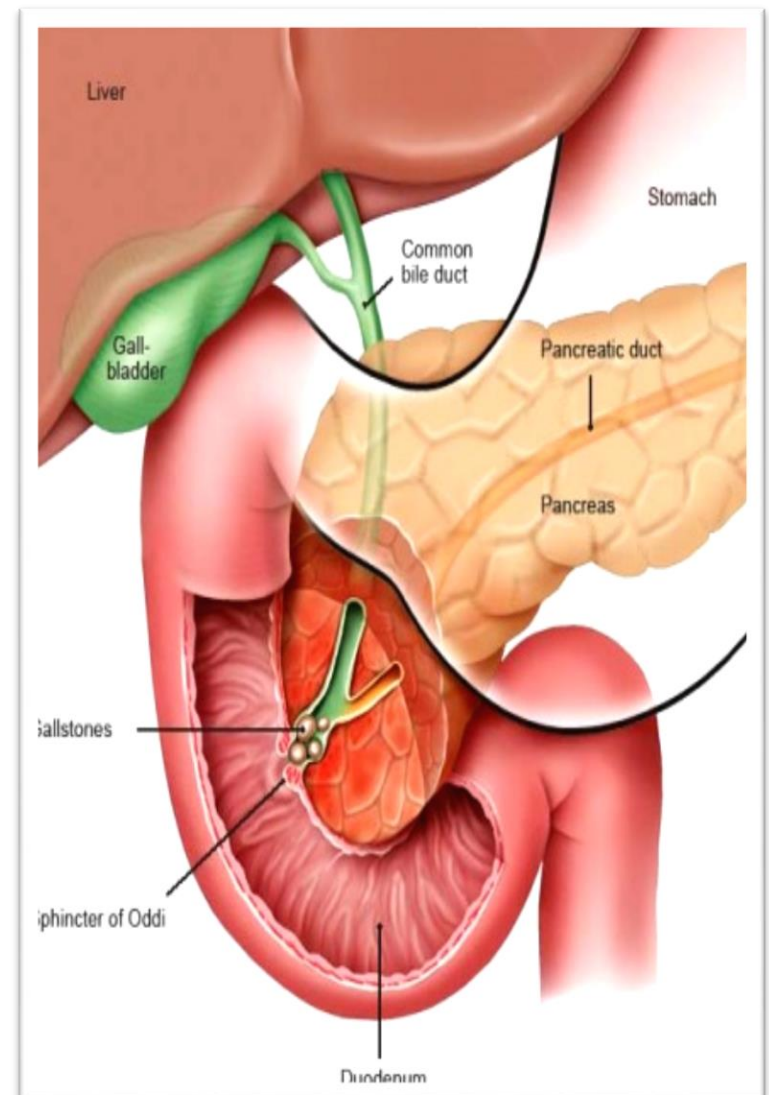


- It is produced by the pancreas in large quantity and secrete them into the small intestine.

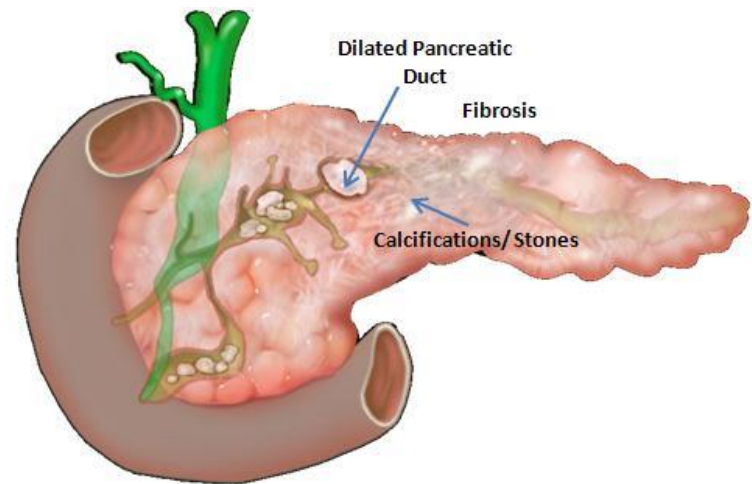
Serum lipase concentration

- The measurement of lipase activity in serum and other fluids evaluate the conditions associated with **pancreas**.
- Lipase concentrations is **increased** in **acute pancreatitis**.
- Acute pancreatitis is a sudden inflammation of the pancreas. Its most common causes are:
 - Gallstones
 - Pancreatic cancer, and other pancreatic disease
 - gallbladder inflammation.

- The common bile duct and the pancreatic duct join together to transport digestive enzymes and bile to the small intestine.
- A gallstone in the common bile duct can cause back pressure in the pancreatic duct leading to pancreatitis **(elevated blood lipase levels)**

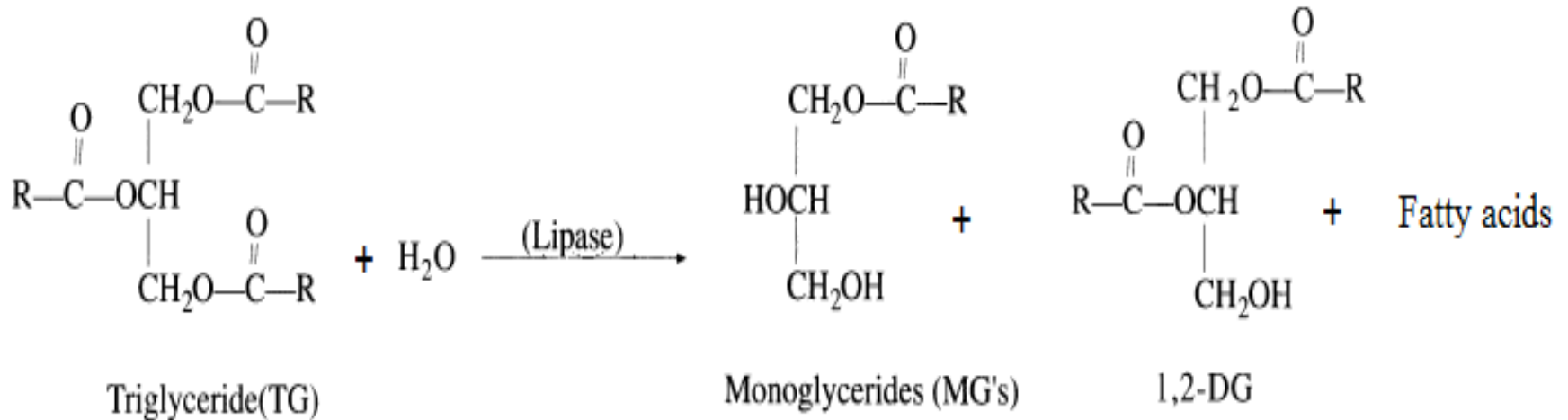


- **A low level of lipase** in the blood may indicate permanent damage to the lipase-producing cells in the pancreas and this can occur in chronic diseases that affect the pancreas such as cystic fibrosis.



Principle of Turbidimetric Method :

- **Serum lipase** hydrolyzes the olive oil emulsion (turbid).
- The decrease in turbidity at 400 nm (after incubation) is proportional to lipase activity in the specimen.



Method:

- **Two test tubes:**

	Test	Blank
Reagent (substrate+ buffer)	3 ml	3 ml
Pre-incubate for 5 minutes at 37° C		
Sample (contains lipase)	0.1 ml	—

- Read the absorbance (A°) immediately at **400 nm** against **distilled water** .
- Then **transfer to water bath** at 37° C and incubate for 5 min then read the absorbance (**A1**) at **400 nm** against **distilled water** .

Calculations:

$$\frac{\text{Test } (A_0 - A_1) - \text{Blank } (A_0 - A_1)}{\text{Blank } (A_0)} \times 3000 = \text{Lipase activity in U/L}$$

- Example:

$$A_1 \text{ TEST} = 0.454$$

$$A_0 \text{ TEST} = 0.464$$

$$A_1 \text{ Blank} = 0.334$$

$$A_0 \text{ Blank} = 0.332$$

$$\text{LIPASE ACTIVITY} = \frac{(0.464 - 0.454) - (0.332 - 0.334)}{(0.332)} \times 3000 = 71.85 \text{ U/L}$$

- Note:

- Reagent blank: if $(A_0 - A_1)$ is a negative value, it should be **considered as zero**. However, it should normally be **between 0.000 and 0.005**.

Normal range:

- In adults: **10-150 U/L**
- In old individuals (more than 60 years): **18-180 U/L**