Triglycerides

By Dr. Yasmine Sami

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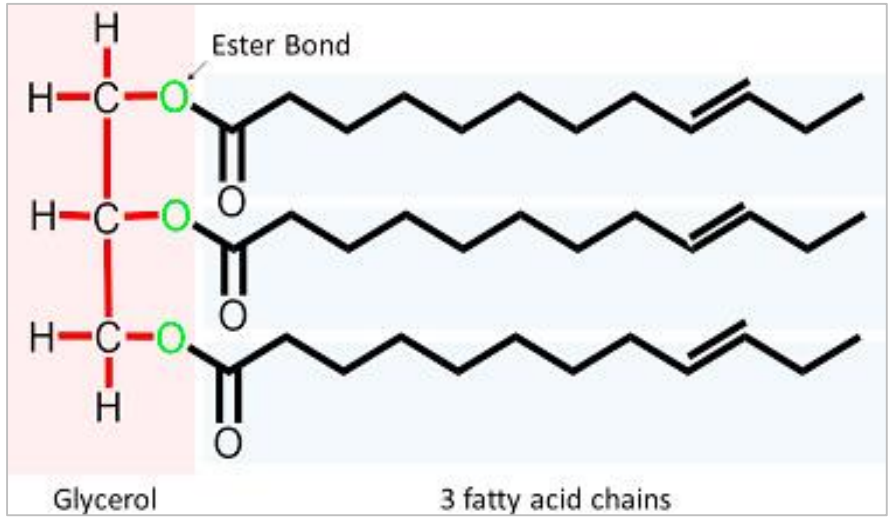
Intended learning outcomes

- Identify the principles of the triglycerides test
- Calculation of TG concentration in the unknown sample

Triglycerides

- An ester of **glycerol** with three **fatty acids**
- Also known as triacylglycerols
- One type of lipid categorized as simple lipid

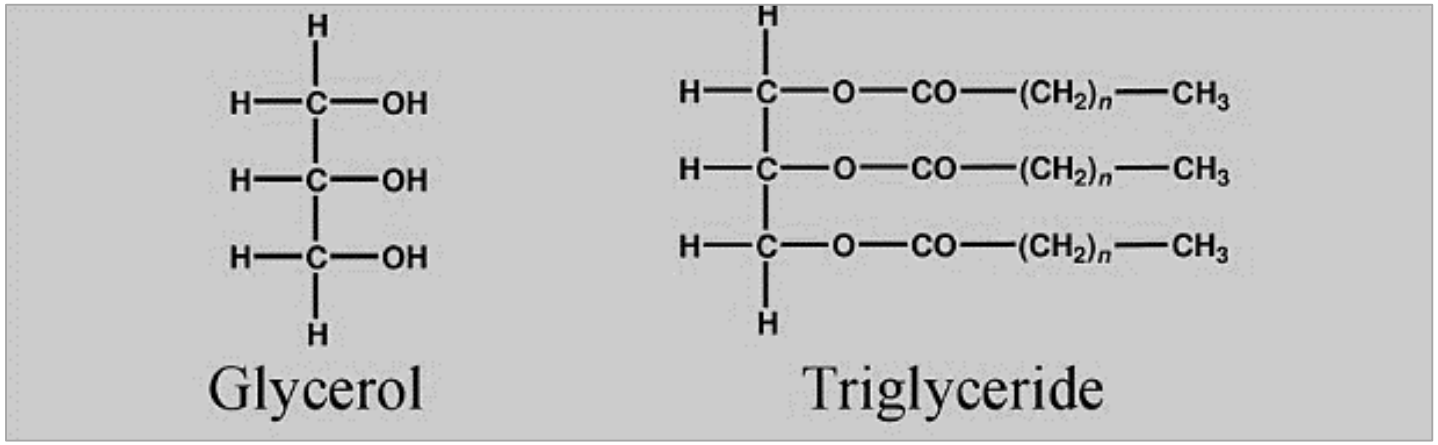


Triglycerides

- Each fatty acid (FA) can be different
- Triglycerides with saturated FA (no kinks)

pack tightly → solid at room temperature

- Triglycerides with unsaturated FA typically oils at room temp



Triglycerides

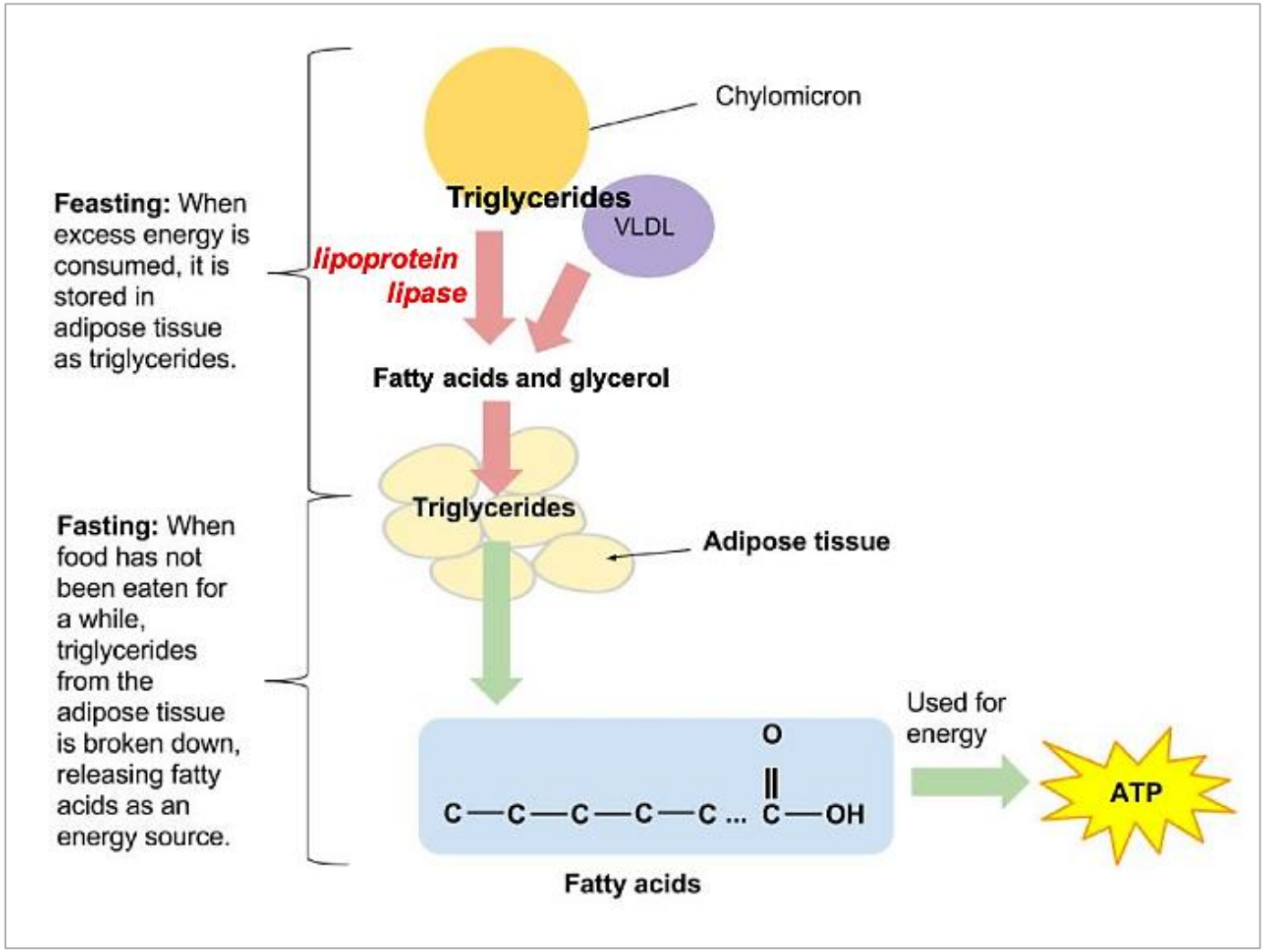
- Triglycerides (TG) represent a concentrated source of metabolic energy and they are a mechanism for **storing energy**
- TG are transported as core constituents of all lipoproteins, but the greatest concentration is in TG-rich **chylomicrons** and **VLDL** particles
- Some TG are needed for good health but high triglycerides can raise the risk of heart disease and may be sign of metabolic syndrome

Formation of Triglycerides

- **Fat and liver cells** cooperate to **synthesize** and store triglycerides
- The high concentration of triglycerides in the blood correlates with the consumption of starchy and fatty food
- Triglycerides cannot pass through cell membranes freely. Special enzymes on the walls of blood vessels (**lipoprotein lipases**) must break down triglycerides into free **fatty acids** and **glycerol.**

Formation of Triglycerides

When the body requires **fatty acids as an energy source** (in case of absence of carbohydrate), the hormone glucagon signals the breakdown of triglycerides by **hormonal-sensitive lipase** to release free fatty acids



Specimen collection and storage

- Fresh, non-hemolyzed serum from fasting patients is recommended.
- TG test needs 12 hours fasting because its level is effected by meal (fatty meal, high carbohydrates meal)
- Triglycerides in serum appears stable for three days when stored at 2-8 ◦C.
- Prolonged storage of the samples at room temperature is not recommended since other glycerol containing compounds may hydrolyze, releasing free glycerol with an apparent increase in total triglycerides content.

Normal range of TG

Level (mg/dl)	Interpretation
<150	Normal range, low risk
150-199	Borderline high
200-499	high
>500	Very high, high risk

Causes of hypertriglyceridemia

There are many reasons why your triglyceride level may be high. Some of them are due to lifestyle habits that increase triglyceride levels. These include:

- **Smoking**
- **Physical inactivity**
- **Being overweight or obese**
- **Increased alcohol consumption**
- **Eating a diet low in protein and high in carbohydrates**

Causes of hypertriglyceridemia

There are also medical conditions that can cause high triglyceride levels, including:

- **Cirrhosis in the liver**
- **Diabetes, especially if it is not well controlled**
- **Genetic factors: as familial hypertriglyceridemia or combined hyperlipidemia**
- **Hypothyroidism**
- **Nephrotic syndrome or kidney disease**
- **Pancreatitis**
- **Drugs as corticosteroids and estrogen**

Principle of Triglycerides measurement

- Measurement of triglycerides is based on determination of Light absorbance by the color compound formed. (**Quinoneimine** formation)
- Forms a Red Color compound
- The **intensity** of the red color is directly proportional to the **concentration of triglycerides** in the sample.
- It is determined by monitoring **light absorbance** at 505 nm

Principle

The enzymatic reaction sequence employed in the assay of Triglycerides is as follows:

Triglycerides + H_2O $\xrightarrow{\text{Lipase}}$ Glycerol + Fatty Acids

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol-3-Phosphate + ADP

Glycerol-3-Phosphate + O_2 $\xrightarrow{\text{Glycerolphosphate oxidase}}$ Dihydroxyaceton + H_2O_2

H_2O_2 + 4-aminoantipurin + 4 chlorophenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine Dye + $2H_2O$

Principle

- The present procedure involves hydrolysis of triglycerides by **lipase** and the glycerol is then phosphorylated
- The glycerol concentration is then determined by enzymatic assay coupled with Trinder reaction that terminates the formation of a quinoneimine dye.

Procedure

Concentration of the standard 200 mg/dl

Set up 2 test tubes as following , and then add:

Reagent	Test	Standard	Blank
Working solution	1ml	1 ml	1 ml
Pre-worm at 37◦C for 2 min and add:			
Serum	10 µl	-	-
Standard	-	10 µl	-

Mix well, incubate at 37◦C for 10min, then the absorbance of quinonimine produced is measured at 505 nm

Concentration of TG

Use Beer-Lambert equation to receive to the concentration of

TG in the patient sample:

Conc. of TG (mg/dl):

$$C_{test} = \frac{A_{test}}{A_{standard}} \times C_{standard}$$

THANK YOU !

ANY QUESTIONS ??

PLEASE ASK