Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 18

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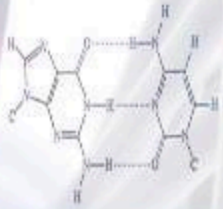
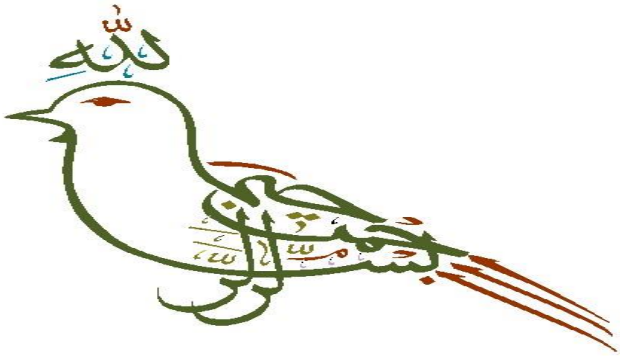
Biochemistry



Majida Al-Foqaraa'



In The Name Of Allah, The Most gracious, The Most merciful

Sheet Outline:

A- Introduction of double bonds into fatty acid , Includes

1- Synthesis of Monounsaturated fatty acid.

2- Formation and modification of polyunsaturated fatty acid

(Continuation of the previous lecture).

B- Biosynthesis of triacylglycerol .

C- Biosynthesis Of phosphoglycerol (cH 17 Lippincott) Introduction.

D- Phospholipids and some examples about phospholipids.

A- Introduction Of double Bonds into fatty acid (Desaturation of fatty acid)

- The process of Fatty acid desaturation involves the introduction of double bonds into the fatty acid in the endoplasmic reticulum .let's start with formation of monounsaturated fatty acid then formation and modification of polyunsaturated fatty acids.

1- Synthesis Of Monounsaturated fatty acid:

■ The most common desaturation reactions in synthesis of monounsaturated fatty acid involve the placement of a double bond between carbons 9 and 10 in the conversion of palmitic acid to palmitoleic acid (16:Δ9) and the conversion of stearic acid to oleic acid (18:Δ9) . (Both have double bond at C9).

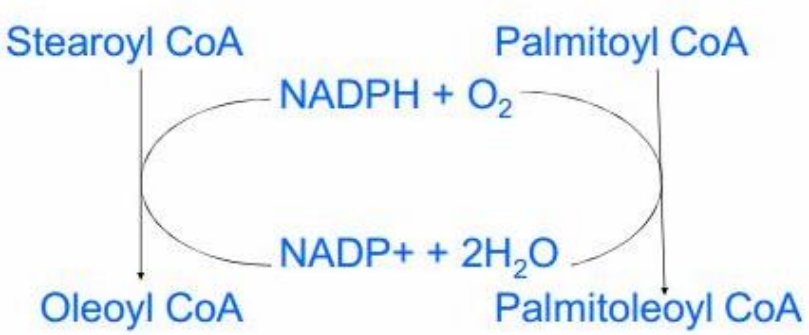
■ The process occurs in the cellular endoplasmic reticulum of human body.

- How monounsaturated fatty acid produced (How the double bond introduced into the fatty acid) ?

Let's take an example:

- The conversion of Stearoyl CoA (18:0) and palmetoyl Co-A (16:00) into the monounsaturated fatty acids Oleoyl CoA (18:1) and Palmitoleoyl CoA (16:1) respectively .Here in both cases we introduce double bond at C9.

Fig (1)



- Double Bonds Introduction mechanism in steroyl Co~A and palmitoyl CoA :-

- Generally, how could be the double bond introduced?

- By addition of oxygen atom At C9 that introduce hydroxyl group into the fatty acid followed by dehydration.(you might ask yourself how could Atomic O introduce hydroxyl (-OH), it is incorporated to the hydrocarbon ,H is already within the hydrocarbon but we introduce the O :P)

- The overall reaction of hydroxylation and dehydration is catalyzed by an enzyme called desaturase or group of desaturases (along with) cytochrome B5 .

- The type of desaturase enzyme required in monounsaturated fatty acid formation in this example is delta 9 desaturase.

- NADPH is required by The enzymes to activate the Oxygen Molecule.(O2)

(Here actually the Dr said, NADPH is required by the enzymes, actually I look for it in MARKs and I found that they enzymes require NADH)

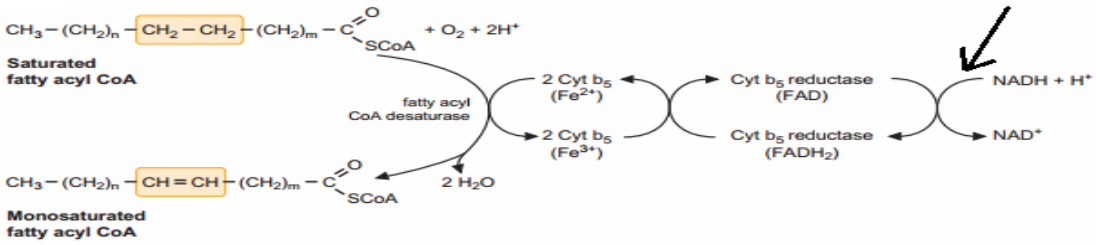


FIG. 33.16. Desaturation of fatty acids. The process occurs in the endoplasmic reticulum and uses molecular oxygen. Both the fatty acid and NADH are oxidized. Human desaturases cannot introduce double bonds between carbon 9 and the methyl end. Therefore, *m* is equal to or less than 7.

■ Desaturation requires molecular oxygen (O2 O-O) as a source of oxygen atom needed in the process to hydroxylate the fatty acid.

■ Firstly, The molecular oxygen (o2) will provide one oxygen atom (one atom will be removed from O2), this atomic oxygen (O) will be reduced into H2o to avoid free radical formation for this reason NADPH is required to reduce this atomic oxygen into water . How is it done?

- ANS/ An enzyme called cytochrome B5 is required to transfer the electrons from NADPH into desaturase enzyme, the latter (desaturase) enzyme will transfer the electron into the oxygen atom (O) and reduce it into water H2O. (This point is not mentioned in this lecture but mentioned in last 2 years recordings :P)

■ Then, hydroxylation takes place .remember we started with O2, one oxygen is reduced into water .The other free oxygen atom (O) is highly reactive so it is added (incorporated) into the hydrocarbon chain of the fatty acid at C9 forming hydroxyl (-OH) at C9.So now we get a hydroxyl derivative of the fatty acid.

■ Finally, Dehydration of the hydroxyl derivative of Fatty acid (H2O will be removed) to produce a double bond (Alkene group instead of ALcohol).
- Why Oleic and Palmitoleic Acids can be synthesized in human cells?
- Because they are monounsaturated fatty acids in which the double bond is introduced at C9 in both of them.

■ No double bonds could be introduced beyond Carbon number 9 in human cells. We can't synthesize fatty acids with double bonds at Carbon 12 or 15. This clears the nutritional essentiality of fatty acids with double bonds beyond (C9) like linoleic and linolenic FA (N.B these are polyunsaturated fatty acids).

- Why we can't introduce a double bond at a carbon beyond C9 while we can add it before? (this one is extra information)

The enzyme desaturase is only able to add double bonds until the site between c9 and c10 but this enzyme lacks the ability to form double bonds from (C-10) to the ω- end of the chain

Monounsaturated fatty acid synthesis main ideas:

1- *It Is done by hydroxylation followed by dehydration*
2- *O2,NADPH,Desaturase and cytochrome B 5 are required in this process.*
3- *In humans, Introduction of double bond couldn't be introduced beyond C9.*

2-Formation and modification of polyunsaturated fatty acid (PUFA):

■ Human can't synthesize PUFAs but can modify them by addition of more double bonds.
■ Modification and formation of PUFA might happen by either elongation or desaturation.
■ In desaturation ,the site of newly introduced double bond must be located 3 carbon before the existed double bond so as the two double bond is separated by CH2

■ Enzymes used in desaturation are desaturases. There are Δ4 desaturase , Δ5 desaturase and Δ6 desaturase.

■ Desaturation of PUFA done in a similar mechanism of monounsaturated fatty acid synthesis (look at figure 2) .

■ The rule Of PUFA Modification (newly introduced Double bond) :

- The double bond must be added between the carboxyl group and the first double bond of The PUFA.

- Let's take the modification of Linoleic Acid to form Arachidonic acid as an example

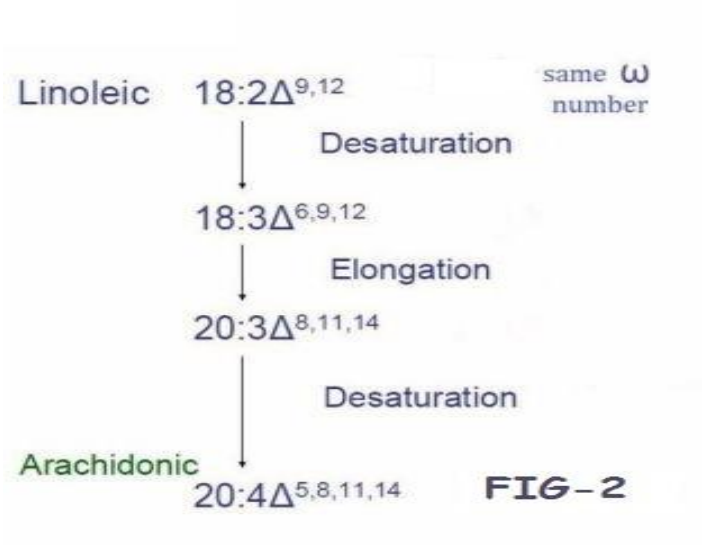


FIG-2

- Linoleic Acid (18:2): It has 18 carbons with 2 double bonds at Carbon 9

and Carbon 12. -humans can't produce this fatty acid because it has a double bond at (C12),so it is an essential fatty acid .It is ω6 fatty acid (Hint 18-12 =6).

* How can we modify it to form Arachidonic acid?

Look at the following steps

1st step: linoleic acid desaturates by adding a new double bond, the new double bond must be introduced three carbon before the first double as the rule of modification indicates. Consequently, delta-6 Desaturase enzyme adds the new double bond at (C6) not C15.The newly synthesized fatty acid is also omega 6. Look at figure 2

-**2nd step** ,Elongation of the newly formed PUFA 18:3 (6,9,12 by addition of 2 more carbons at the carboxyl group side, it will produce a fatty acid has 20 carbon with 3 double bonds located at 8,11and 14 (20:3 Δ 8, 11, 14). The original Double bond locations is not really changed upon elongation, they are relatively changed as there is 2 more carbon is added. The omega classification is still the same ω6.as there are 2 carbons added; carbon no.1will be Carbon no.3 and new carboxylic group will be added (carbon no 1).

(18+2=20) Δ 6+2=8,9+2=11,12+2=14. (C+2Δ Double Bonds +2)

3rd step :Another desaturation of (20:3 Δ 8 ,11,14) Fatty acid will be occurred at C5 producing Arachidonic acid (20:4 Δ 5,8 ,11,14) .

- The Dr didn't mention the name of the FA we start with in the second step and it is not the same of linolenic acid we did take in biochemistry 1 cause that one was with double bonds at (9,12,15) not (6,9,12).actually I got its name from Wikipedia and it is called gamma linolenic acid . in addition, the fatty acid we start with in 3rd step called eicosatrienoic acid (also from wiki).

Formation and Modification Of PUFA Main ideas:

- Modification take place by elongation and desaturation.
- *The rule of PUFA modification :*

The double bond must be added between the carboxyl group and the first double bond.

- In desaturation :

The site of the newly introduced double bond must be located 3 carbon before the existed double bond

- *In elongation we add the number of carbon introduced to the original number of carbon units and the location no. of the double bonds.*
- *The Omega classification does not change upon modification, because modification does not take place from the omega side.*

Biosynthesis of triacylglycerol & phosphoacylglycerol

Introduction:

-TAG structure: Triacylglycerol: it consists of a glycerol esterified to 3 fatty acids.

-Phosphoacylglycerol structure: It consists of a glycerol esterified to two fatty acids but at the last carbon (c3) it is esterified to phosphate which in turn can form an ester bond with alcohol.(phosphate forms 2 ester bonds (diester) with alcohol and glycerol.

-You can observe the huge similarity between the 2 structures in which both of them has a glycerol esterified to two fatty acids. Therefore, the biosynthesis of both are quiet similar. (Actually in early steps bas yalla) .look at fig 1.

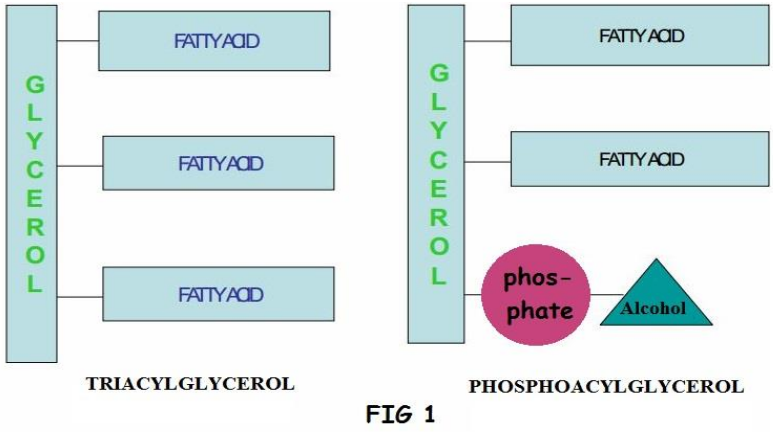


FIG 1

- Phosphatidic acid is a common intermediate in both TAG and Phosphoacylglycerol biosynthesis.
- Phosphatidic acid structure: it consists of a glycerol esterified to two fatty acids and esterified to phosphate at C3. Look at fig2

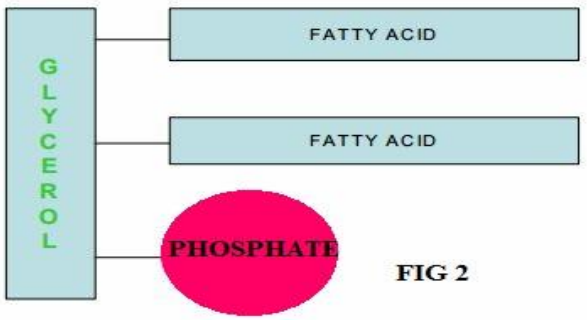


FIG 2

**Biosynthesis of Triacylglycerol

- **Introduction** :

■ Biosynthesis of TAG requires acyl~CoA (the active form of FA) and glycerol phosphate so it is not simply joining fatty acid with glycerol. There is no enzyme that can join fatty acid with glycerol directly.

■ The fatty acid must be in the active form (Acyl Co~A). Why active form is required, what is the importance of fatty acid activation in TAG synthesis?

- Because FA active form contains a thioester bond yields high energy once broken, but if you want the full explanation look at the next point.

-Hydrolysis of triacylglycerol forming Diacylglycerol and fatty acid is an exergonic reaction (ΔG= –ve➔ exergonic).Hydrolysis requires the enzyme lipase.

Hypothetically, let's assume the inverse of the first reaction is going ,Adding Fatty acid is to Diacylglycerol , the reaction wouldn't proceed forward because it will be endergonic ΔG= +ve (not favorable)

What is the solution for this problem?

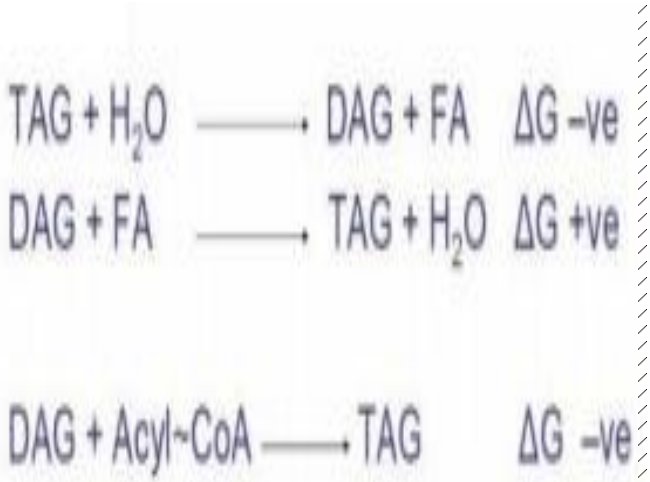


FIG 3

-Donation of a Fatty acid from an activated fatty acid as acyl Co~A will be the practical solution for this problem ,it will react with diacylglycerol , the reaction is accompanied by cleavage of the high energy thioester bond that transfers the fatty acyl into Diacylglycerol forming Triacylglycerol. This reaction will be exergonic (ΔG= –ve) . Look at fig 3

-Finally, the acyl group can be transferred only if the donor of carboxylic acid (carboxyl group to form esterification reaction) is Acyl CoA (active form).

■ Now, How acyl co~A is produced?

- Conversion of fatty acid to acyl co~A requires thiokinase which in turn requires an equivalent of 2 ATP because ATP is converted to AMP. (The same first step in Beta oxidation of fatty acid)

FA + CoA + ATP ➔ (acyl-CoA)+ AMP + 2Pi

AMP + ATP ➔ ADP + ADP

- Now we knew how can we obtain the acyl co~A and we knew that biosynthesis of Triacylglycerol requires glycerol-3-phosphate. now how can we obtain glycerol-3-phosphate ?

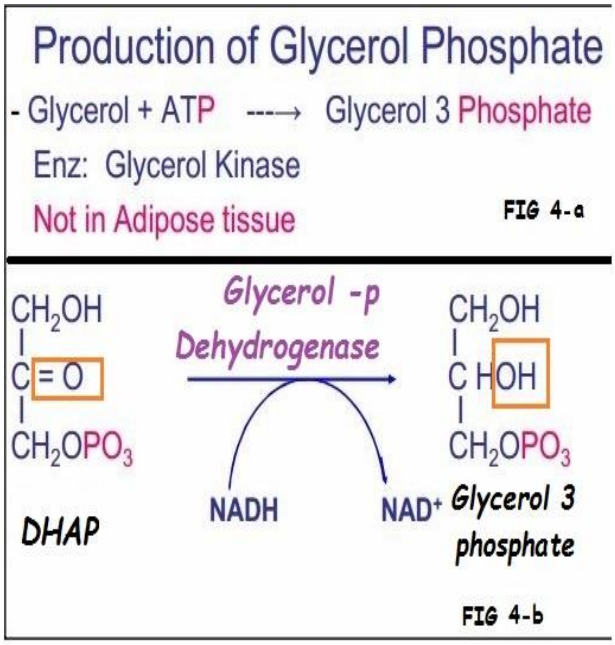
***Production of glycerol- 3- phosphate:**

-Let's retain to our question, how can we obtain glycerol-3-phosphate?

■ Glycerol- 3- Phosphate can be produced by two methods:

1st method :phosphorylation of glycerol using glycerol kinase (In liver only):

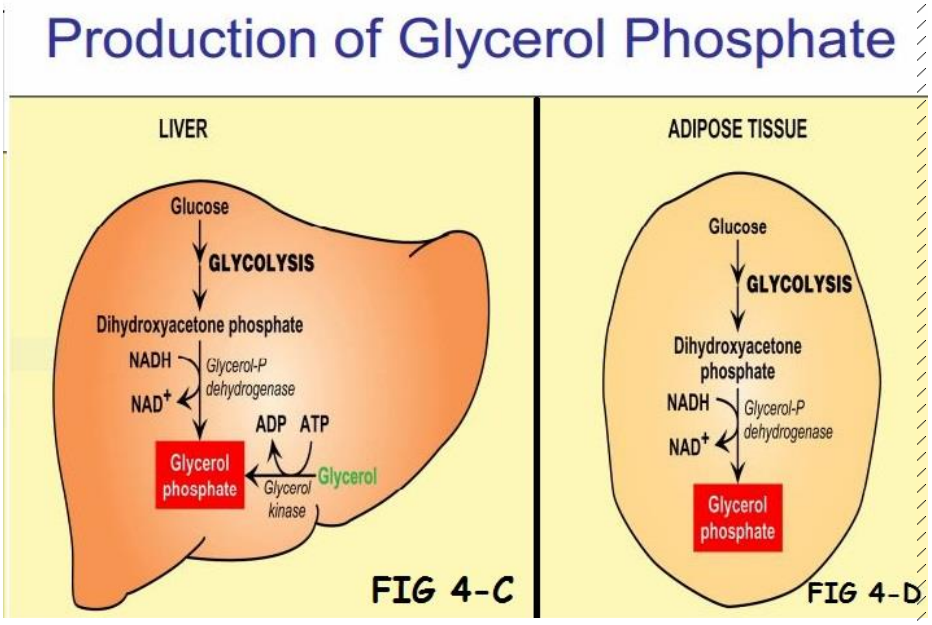
Phosphorylation is done by an enzyme called Glycerol Kinase. Glycerol-3 P is produced by transferring the phosphate group of ATP to glycerol at position #3. Look at the equation in Fig 4 a (above the line).



2nd method: Using of glycolysis intermediates, i.e. Dihydroxyacetone-p :

(IN Liver And adipocyte) (FIG 4-b,c)

Reduction of DHAP (Dihydroxyacetone –p) done by reduction of to G3P by glycerolphosphate dehydrogenase accompanied with the oxidation of NADH to NAD+. (As illustrated in the equation Fig 4b).



*look at fig 4-D the following info is about it:

- This is an adipocyte, where TAG is stored, comprising 90% of its total volume.

-TAG is hydrolyzed in response to Hormonal signal producing 3 FA and glycerol. Fatty acids and glycerol leave the adipocyte toward different tissues Hormonal stimulation Of TAG hydrolysis represented by increasing level of Glucagon and epinephrine. (You all knew that increase level of Glucagon means low blood glucose).

-Adipocyte lacks Glycerol Kinase enzyme, Glycerol -3- phosphate can't be produced by simple phosphorylation using Glycerol kinase as in the liver, so that the intermediate of glycolysis "DHAP" exploited to generate glycerol 3 phosphates.

- When there is high blood glucose, high insulin, expression of glucose transporter protein GLUT-4(insulin dependent transporter) is increased on adipocyte membrane enhancing Triacylglycerol synthesis and storage.

* In the cause of diabetic patient they may come to the hospital because of losing body weight, why? Because there body does not secret insulin so glucose will not enter the adipose tissue through GLUT-4 transporter, so the synthesis of TAC (fat) will became less which causes their body weight to reduce.

-Although adipocyte lacks glycerol kinase, adipose tissue is the most active tissue in Triacylglycerol production. Glycerol kinase is not expressed at the adipose tissue.

* What is the importance of glycerol kinase being absent in the adipocyte?

- Hypothetically, if glycerol kinase is present in adipocyte, glycerol will be rapidly phosphorylated into glycerol 3 phosphate going in TAG synthesis at the same time of degradation. The cycle will be continued because fatty acid would be converted to fatty acyl Co~A and the glycerol- 3- p is available. If there is a continuous cycle of synthesis and degradation, nothing will be achieved other than energy wasting in form of 2 ATP molecules being consumed during FA synthesis. Absence of glycerol in adipocyte is crucial to prevent energy wasting.

- I think now we have glycerol-3P and acyl CoA .6aiyeb let's go to biosynthesis! yalla

Triacylglycerol (TAG) Biosynthesis Pathway : (refer to FIG 5)

1st step: The acyl group is transferred to be esterified at carbon #1 replacing the hydroxyl group in Glycerol 3 phosphate by an enzyme called acyltransferase forming 1-acyl-3-phosphoglycerol (aka. Lysophoshatidic acid). This is a transfer reaction, so that we call the enzyme acyltransferase.

2nd step : one more acyl group transferred to be esterified at C #2 in 1-acyl-3-phosphoglycerol forming phosphatidic acid. This transfer reaction is also catalyzed by acyltranferase (The common intermediate in TAG & phosphoacylglycerol biosynthesis)

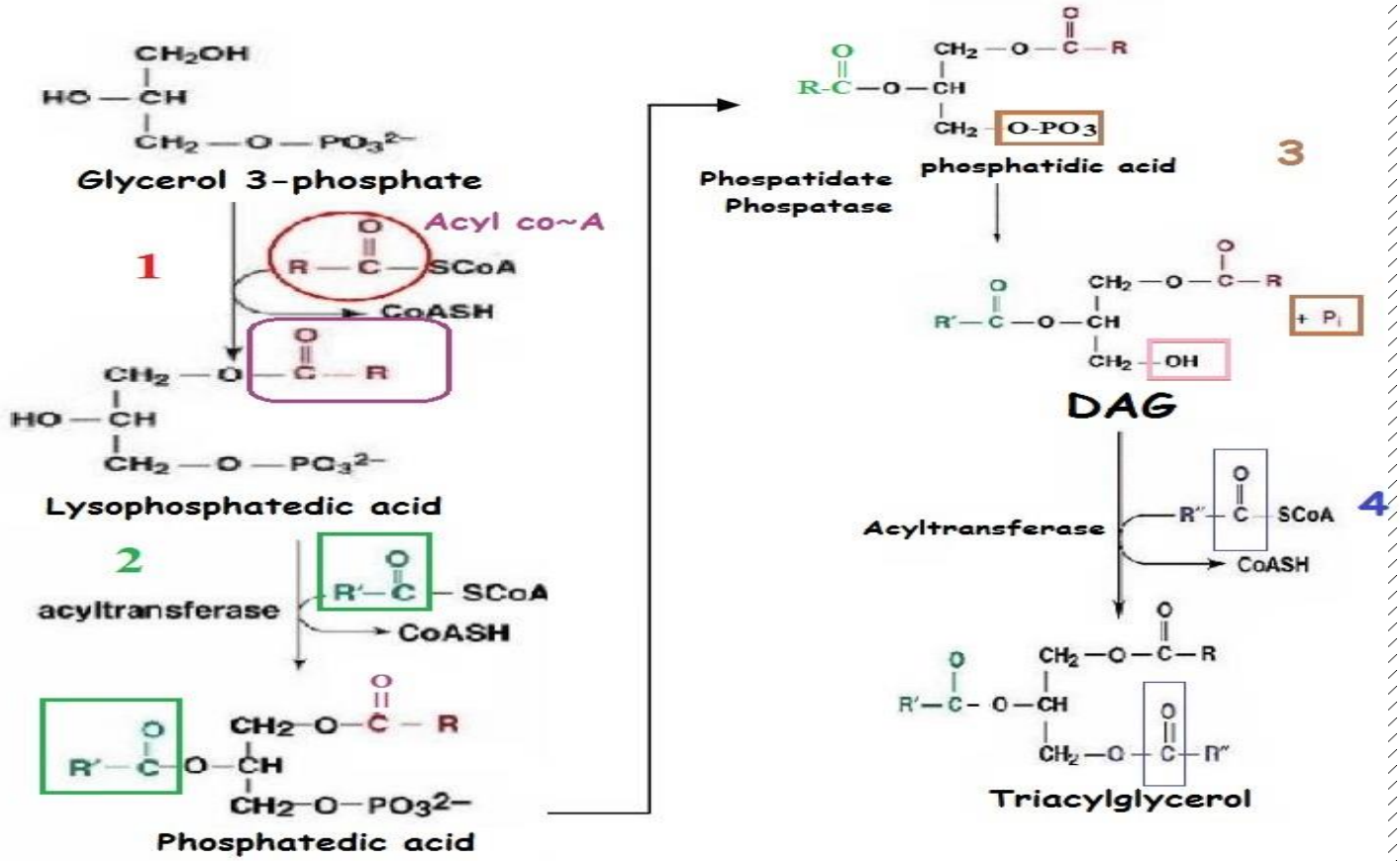
Take a breath! Look at phosphatidic acid, what's remaining to get the TAG?

2 things: 1- phosphate group removal then esterification of other acyl group at C#3 forming triacylglycerol. Let's continue

3rd step : Removing of the phosphate group from phosphatidic acid forming DAG, ,this step is catalyzed by phophatidic acid phosphatase. (Phosphatidate phosphatase)

4th step: A third acyl group will be transferred from acyl co~A a to Diacylglycerol forming triacylglycerol.

(FIG 5 ↓)



**** Biosynthesis of phosphoacylglycerol :**

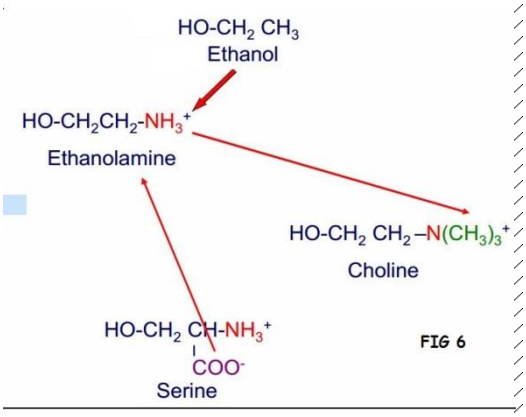
(AKA glycerophospholipid, phosphoglycerides) :

-Structure of phosphoacylglycerol (look at fig 1 above in the previous topic)

- We already knew its structure which is a glycerol backbone esterified to two fatty acids and at C3 it is esterified to phosphate group which is further esterified to an alcohol.
- We knew the structure of phosphatidic acid (Fig 2 in the previous topic) , it consists of a glycerol esterified to two fatty acids and also esterified to phosphate at C3. It is a common intermediate in TAG and phosphoacylglycerol synthesis.
- phosphatidic acid can form an ester bond with alcohol. 5 examples of alcohols were discussed in the lecture, three of them contain amine group which are serine, ethanolamine, choline and the other two are inositol and glycerol. When these compounds form an ester bond with phosphatidic acid, we'll call it Phosphatidyl-(anyone of those 5 alcohols).

- Example, Phosphatidyl-Serine or Phosphatidyl-Inositol. A good thing to remember is that the suffix –ic is replaced with -yl as in Phosphatidyl, the suffix -yl indicates that the acid is a part of an ester bond (forming an ester bond).

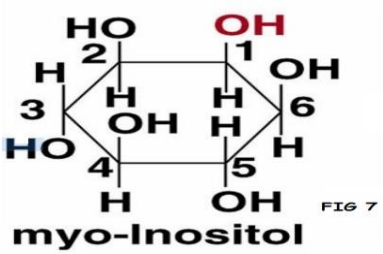
■ Replacement of one hydrogen in ethanol with an amine group produces ethanolamine, this doesn't mean this is how ethanolamine is synthesized in the body and doesn't mean that ethanolamine is converted to Ethanol, this is just a simple way to show you the relations between the structures to memorize them :'(.



■ Decarboxylation of serine by removing carboxyl group produces ethanolamine. In addition replacement of the 3 hydrogen groups in ethanolamine at the nitrogen atom with 3 methyl groups gives us choline (Methylation Of Ethanolamine). (look at Fig 6)

■ N.B Ethanolamine, Choline and Serine all contains a positive charge, because they are amines .always when nitrogen bonded to 4 atoms, it will be positively charged.

■ Myoinositol, six-membered ring (a cyclohexanol ring). It contains only hydroxyl group with no amine groups. (Fig 7).



* Examples Glycerophospholipids (glycerophosphlipids = Glycerol based phospholipids)

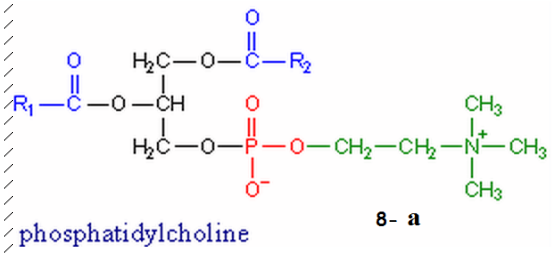
(Phosphatidyl choline,phosphatidyl serine,phosphatidyl inositol and phosphatidyl glycerol

A-Phosphatidyl choline AKA lecithin

■ It contains a hydrophobic and hydrophilic part that means it is an amphipathic molecule. It acquires its hydrophilic property by the presence of nitrogen carrying a positive charge and an oxygen carrying a negative charge .The region that contains these both charged atoms considered as the hydrophilic region (the Green and red parts in Fig (8 –a) while the uncharged hydrocarbon portion is the hydrophobic region. Biochemists use the space-filling model to visualize the shape of the molecule and to find out its properties (FIG 8-b). The kink in the structure of phosphatidyl choline is due to the presence of a double bond. The red balls represent oxygen atoms which are polar (one of the oxygen atoms at the phosphate group bears a negative charge). The yellow ball is phosphate.

■ Once lecithin dissolved in water, the hydrophobic region will become close to each other and the hydrophilic region will face the aqueous. By this mechanism lipid bilayer, liposome and micelles can be formed. Micelles are very small spheres with a hydrophobic interior and hydrophilic surface .Fig 8-(c-a) (the one at the left is micelle, (c-b) the one in the middle is a membrane bilayer,(8-c) the one at the right is liposome which is also a membrane bilayer.

■ Phospholipids work as an emulsifying agents (mixing of water-insoluble substances with water). E.g if we get a test tube containing lecithin in the form of micelles and mix it with oil and water, oil will go into the interior part of micelles where a non-polar hydrophobic environment is there. That's why lecithin, a phospholipid , is put in chocolate and powder milk, to allow mixing of the fat present within these substances with the watery medium of our gastrointestinal tract. Else, dissolving will occur with extreme difficulties.



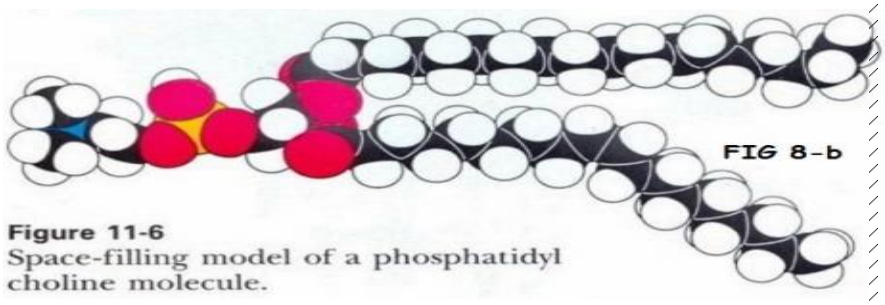
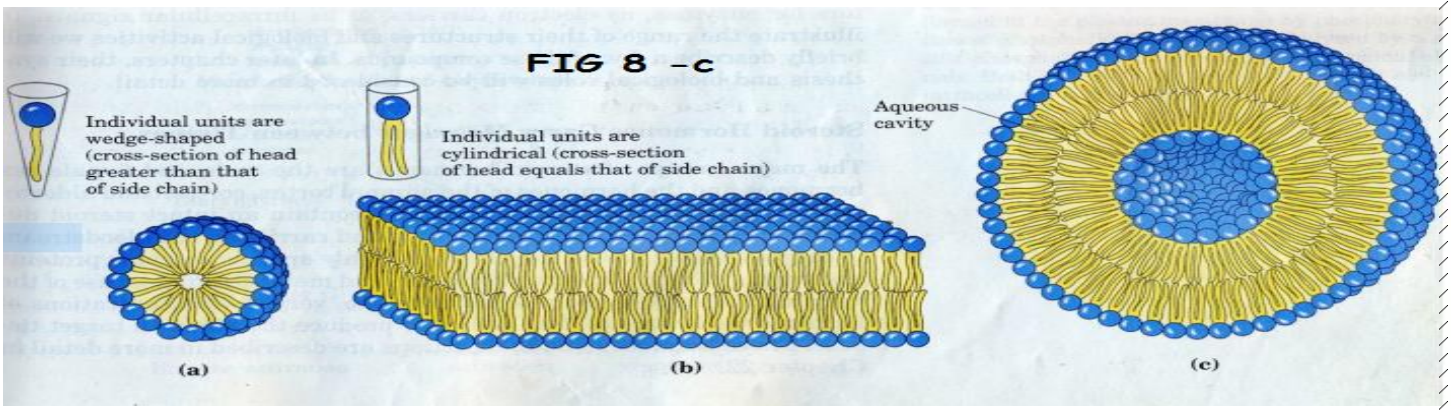


Figure 11-6
Space-filling model of a phosphatidyl choline molecule.

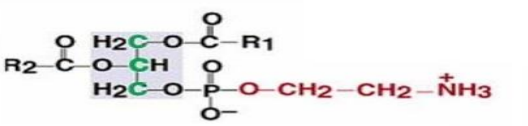


In Figure (9-12) you should recognize these structures of different phospholipids

-B-Phosphatidyl Ethanolamine. C –Phosphatidyl serine ➔these 2 phospholipids characterized by two long hydrocarbon chains and polar head (bearing + & - charges)(amphipathic molecules). (Look at Fig 9)

-D) Cardiolipin: two molecules of phosphatidic acid connected through glycerol. Cardiolipin is found in membranes (especially the inner mitochondrial membrane IMM).Fig

- Phospholipids are components of the plasma membrane, phosphatidylserine ,*phosphatidylethanolamine* and phosphatidyl serine can be found in .



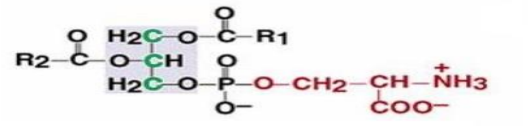


FIG 9

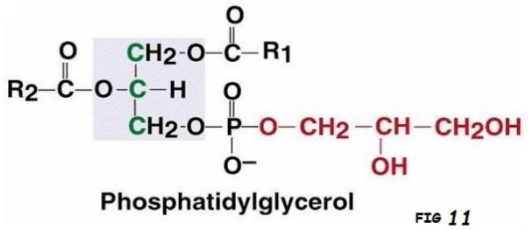


FIG 11

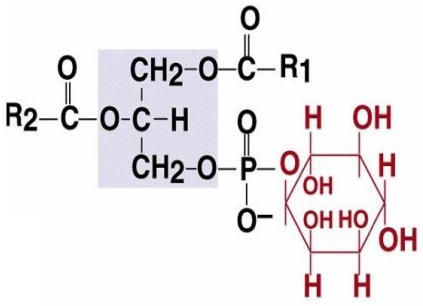


FIG 10

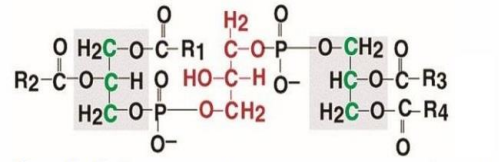


FIG 12

** Degradation of phospholipids :

- Phospholipids can be degraded by hydrolysis of the ester bonds
- There are four ester bonds in phospholipids.
- Each ester bond has its specific enzyme, because ester bonds have different locations.
- These enzymes called phospholipases, All of the following 4 phospholipases work on an intact phospholipid molecule, these are :

1- Phospholipase A1:it breaks the ester bond between the glycerol and the first fatty acid.
2- Phospholipase. A2:it breaks the ester bond between the glycerol and the second fatty acid producing lysophosphatidylcholine
3- Phospholipase C:it breaks the ester bond between the glycerol and the phosphate group.
4- Phospholipase D: it breaks the ester bond between the phosphate group and the alcohol.

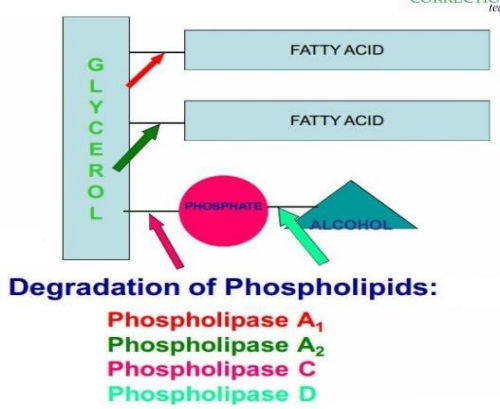


FIG 14

■ These 4 Enzymes don't work in sequence, no any enzyme of them work after the other.
- E.g. The enzyme (Phospholipase A2) does NOT act after A1
As the name may imply, it actually acts on an intact molecule.

■ There is a unique type of phospholipase called phospholipase B. It acts on the product of phospholipase A2 (lysophosphatidyl"something") by removing the first fatty acid. (e.g. "something" could be choline e.g Lysohphospatidylcholine :P)
■ It is more accurate to name Phospholipase B as lysophospholipase because it does NOT act on an intact molecule).
■ Lysophosphatidylcholine (LPC) causes lyses of the cell membranes, that's why it's a lysophospholipid and considered to be a strong detergent.
■ Lysophosphatidylcholine acts as an active surfactant, surface tension-reducing agent.

Surfactant definition from wiki,

It is a compound that lowers the surface tension between two liquids or between a liquid and a solid. Surfactants may act as emulsifiers and detergents

** Phospholipase A2:

■ It is present in many mammalian tissues.
■ It is found in snake venoms. So if someone is bitten by a snake PL-A2 will act at the bite site reaching the phospholipids of the plasma membrane hydrolyzing them to lysophospholipids which will cause cell lyses.

- Just read the info about other phospholipases .from this photo

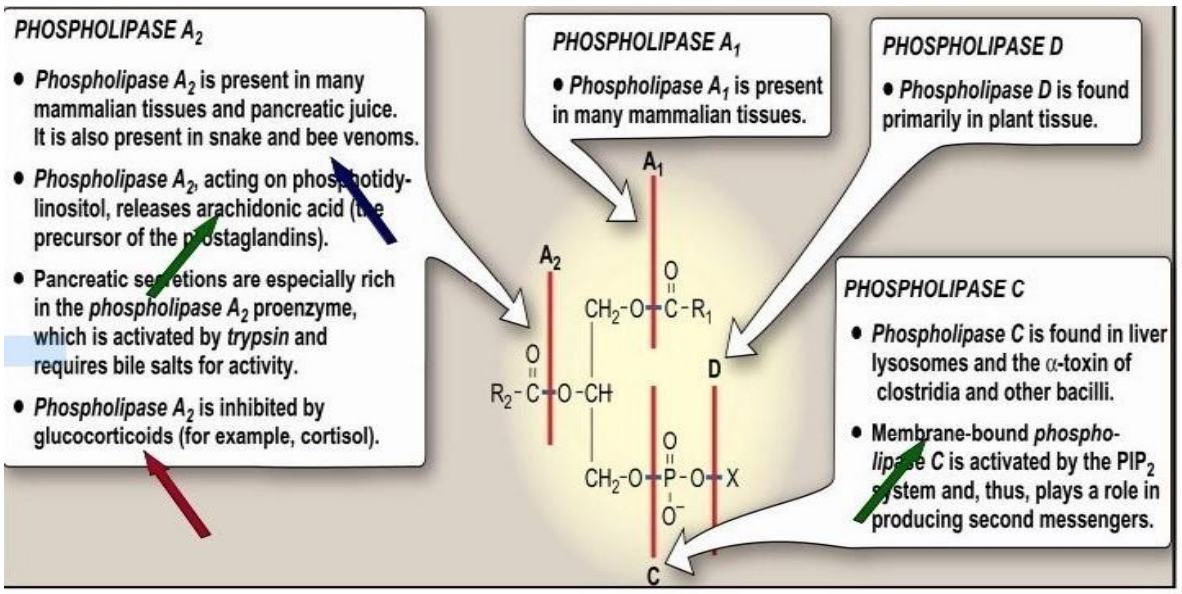


FIG 16

- N.B. PLA2 won't be found in Suarez Saliva.(just kidding , Please boys never tell girl classmates who is Suarez ,just tell them he is not a good singer ;) :P . .Warning , girls if you got a so inquisitive personality to google " who is Suarez " you might find an inappropriate violent content ☺

- (never look at Fig 17 it is not important at all .

** here is a past paper questions repeated every year : P

-what is correct about this F.A 20:5 delta 5,,8,11,14,17 (Final 2012,2011) the answer was: -it is an essential fatty acid

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