Sheet ○Slides

I

Subject :	Regulation of Synthesis
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Number :	23

In this lecture we'll talk about regulation of fatty acid synthesis and oxidation . Both processes cannot occur at the same time because this will result in the loss of energy, so we have to regulate them.

Regulation of Synthesis:

1) *Regulation of Acetyl CoA carboxylase*: it should be regulated since it's the 1st step of fatty acid synthesis, to prevent synthesis when it is not needed:

A) Allosteric mechanism (rapid)

B) Phosphorylation, which leads to inhibition.

Think about Glucagon/Epinephrine→both bind to Gsα→increase cAMP→cAMP dependent protein kinase A activation→Phosphorylation. And this makes sense by knowing the function and the time at which Glucagon is secreted (when no food→we have to burn fat not make fat).

***2) Regulating the amounts of the enzymes*:** If synthesis is not necessary or not possible right now, why to make the enzymes of the synthesis?!
**Now the oxidation is regulated by:

1) Supply of fatty acid: which is controlled by hormones, because as we said before, the hormone sensitive lipase regulates mobilization of fatty acids. When the hormone sensitive lipase is activated, the amount (supply) of fatty acids is increased, simply due to mobilization of fat in the adipose tissue. When there's high amount of fatty acids, this stimulates fatty acid oxidation (a lot of substrate)

2) The availability of NAD+: if NADH levels are high, this means that NAD+ levels are low; because the sum of both NAD+ and NADH in the cell is constant (fixed), and NAD+ is required for oxidation, so if NAD+ levels are low→oxidation will not occur. This is another example regarding substrate availability.

3) Entry to the Mitochondria is regulated: entry of Acyl groups into the Mitochondria requires Carnitine shuttle.

Note: that **malonyl CoA** which is produced during fatty acid synthesis indicates that fatty acid synthesis is active so it inhibits the entrance of fatty Acyl CoA into the mitochondria by inhibiting Carnitine (thus without entrance they will be no fatty acid oxidation). By knowing that, notice that when fatty acid synthesis is active, fatty acid oxidation is inactive. This is very important to prevent simultaneous oxidation and synthesis of fatty acids.

Note: malonyl CoA is produced from acetyl CoA by ACC (acetyl CoA Carboxylase).

- Now, what do you think is an advantage of fatty acid oxidation? What does it lead to?

  It's important to decrease TAG thus decreasing **obesity**. So one of the methods by which we can solve the problem of being overweight is to enhance/stimulate fatty acid oxidation. By knowing that many of the drugs used by humans are enzyme inhibitors rather than stimulators (there are no enzyme stimulating drugs because the enzymes in our body are normally active, if you want to manipulate the enzyme you inhibit it, and you cannot create maneuvers to increase enzyme activity, mostly). Based on this, if you want to give a drug to decrease obesity, what will you give? Will you give fat-burning enzyme stimulator? We said that this is not possible, so think about it the other way, and give a drug that inhibits fatty acid synthesis, mainly to inhibit ACC enzyme→preventing formation of malonyl CoA→STIMULATE OXIDATION. So it's simply by inhibiting the inhibitor, which is malonyl CoA.

- Side note: a group of scientists tried to introduce mutations to ACC in genetically engineered mice, in order to decrease obesity. They found that these mice eat 40% more than normal mice, and on the contrary, weigh 15% less! So this subject is under continuous research.

- **Elongation of FATTY ACID**:
  Elongation of fatty acid more than 16 carbons (produced by fatty acid synthase) is done in *Endoplasmic Reticulum*, because it's hydrophobic. This process is done using similar sequence of reactions (in FA synthesis) but different enzymes because fatty acid synthase is only specific for 16 carbons.

- The sequence of the reaction is <u>as usual</u> : Acyl CoA + malonyl CoA together in ER produce Acyl CoA by several steps include using of 2NADPH , the purpose is to produce fatty acid longer than 16 carbons

➢

Malonyl CoA + Acyl CoA(n)

↓

↓ 2NADPH + H+

↓

↓ 2NADP+

Acyl CoA (n+2)

n = 16 or more carbons

- Note: elongation of *short chain* fatty acids (4-8 carbons obtained from milk or butter…) occurs in the mitochondria.
- In the mitochondria, similar reactions to the **oxidation** but in the reverse direction (3 of the 4 enzymes used in oxidation can be used in the reverse direction for elongation). It's reversed except for the step that use FAD (Acyl CoA dehydrogenase, this is irreversible)
  which we overcome by using NADPH as electrons donor.

- **Introduction of double bonds (dbs = double bonds)**

A) Synthesis of monounsaturated fatty acid:
(e.g. oleic acid(18:Δ□) and palmitoleic acid(16:Δ□).

- Occurs in ER in human cells. Note that humans can't introduce a Double bond **beyond** carbon 9-we can introduce double bonds before c9 and on c9 but not after that-(usually the double bond is at carbon 9), so we cannot introduce double bonds at carbon 12 or 15, rather, these fatty acids (like lenoleic and lenolenic acid) must be taken from the diet, so these are **essential** fatty acids.
- It's not done by just removing 2hydrogens. It's by introducing a hydroxyl group, followed by removal of a water molecule (dehydration).

- Take stearic acid (18 carbons saturated) that can be converted into oleic acid (18:1Δ9).
  Stearic acid (must be active→bound to acetyl CoA (being stearoyl CoA)). Same thing happens to palmitic acid.
- Two oxygen atoms (O2) are needed. One oxygen atom will be introduced as hydroxyl group, while the other oxygen is going to be reduced by NADPH (which is oxidized) to make H2O.
- The enzyme is called <u>Δ**9** **desaturase**</u>: which contains <u>cytochrome b5</u>, the cytochrome is involved in the transfer of electrons.

- Δ9 desaturase can introduce double bond at carbon 9.
- So NADPH is needed although we are removing hydrogens, in order to reduce the oxygen, while the other oxygen is added to the fatty acid. So by this we produce one water molecule, and then when dehydration of the fatty acid occurs, another water molecule is removed and a double bond is introduced at c9 making stearoyl CoA becoming oleoyl CoA and palmitoyl CoA becoming palmitoleoyol CoA, respectively.

- Now, double bonds can be introduced to already unsaturated fatty acids (we can add more double bonds). Where can we add this new double bond?
  Between the <u>carboxyl group and the already existing db</u>.

  *Note: this new db must be added to a carbon which is 3 carbons less than the carbon holding the db (so if the old db is on c9, and since we can only add before that- between the carboxyl group and the carbon of the db- so the new db will be added on c6, because dbs are non- conjugated, they are separated by methylene(CH2) groups and look like this: double then single then single then double then single then single and so on).
- We can add dbs at carbons 4 or 5 or 6 according to the already existing db.

- <u>*Modification of polyunsaturated fatty acids:*</u>
  by adding more dbs or adding more carbons (elongation).

- Example is: Lenoleic acid (**18:2Δ9,12**). If we want to add a db (type of modification is: <u>desaturation</u>), where is it going to be? On c15?.....**NO**, rather, we introduce the db on carbon 6 so yield (18:3Δ6,9,12).

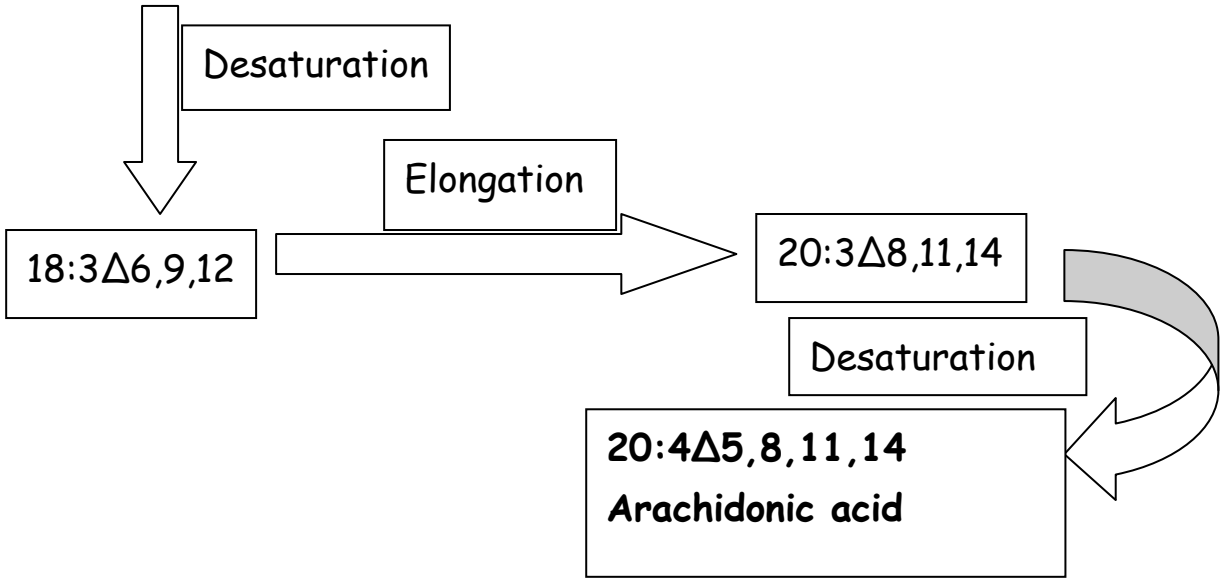
  *Note: what is the ω classification of 18:2Δ9,12?
  →ω6. What about 18:3Δ6,9,12? It is also ω6. Because the relation between the ω-carbon and the db nearest to it has NOT changed.
- Now, as for 18:3Δ6,9,12 we can elongate it (add 2 more carbons). What is the product of elongation of this fatty acid?
  Since we will add 2 carbons, the total number will be 20 carbons, but what about the position of the dbs? Since we add the 2 carbons **on the carboxylic carbon** so the position of the db will

differ. Each db is now 2 carbons farther to carbon number 1 so we add 2 to each db position to yield 20:3Δ8,11,14. But the ω classification hasn't changed (the distance between the last db-if you count from the carboxyl-and the ω carbon has not changed), so it is still ω6.

- If we do another desaturation, what will be the outcome? It will be **20:4Δ5,8,11,14** →this is Arachidonic acid (which is still ω6).

**Is Arachidonic acid essential or non-essential fatty acid? BOTH ARE RIGHT. Because if food contains limited amount of lenoleic acid, the body won't utilize it to make arachidonic acid, which becomes essential fatty acid that must be taken from food, but if the diet is rich in lenoleic acid, your body can make arachidonic acid, so it's also non-essential. So asking such question is non-sense in itself!!

Linoleic acid 18:2Δ9,12



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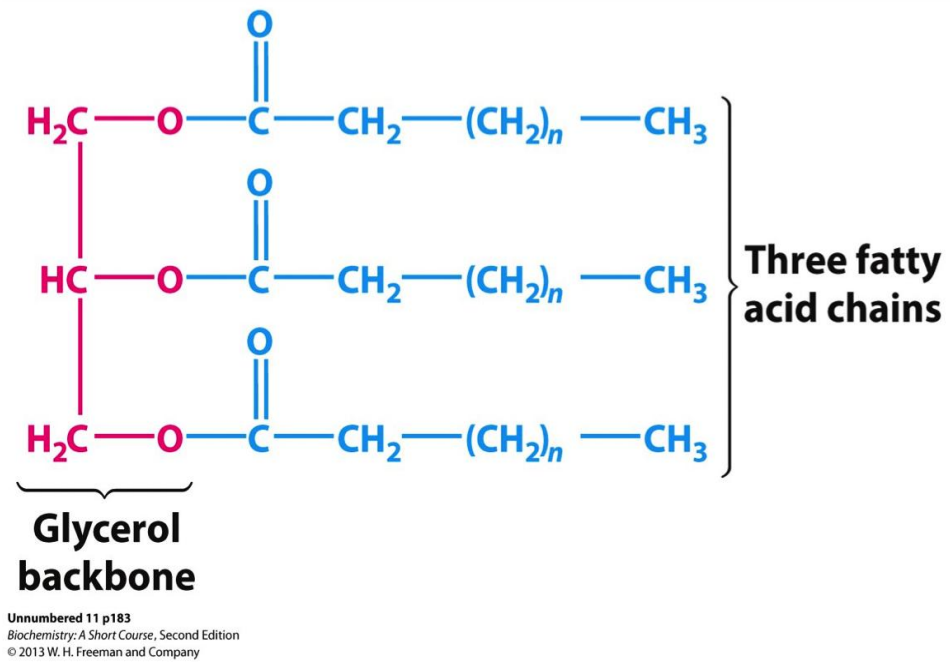
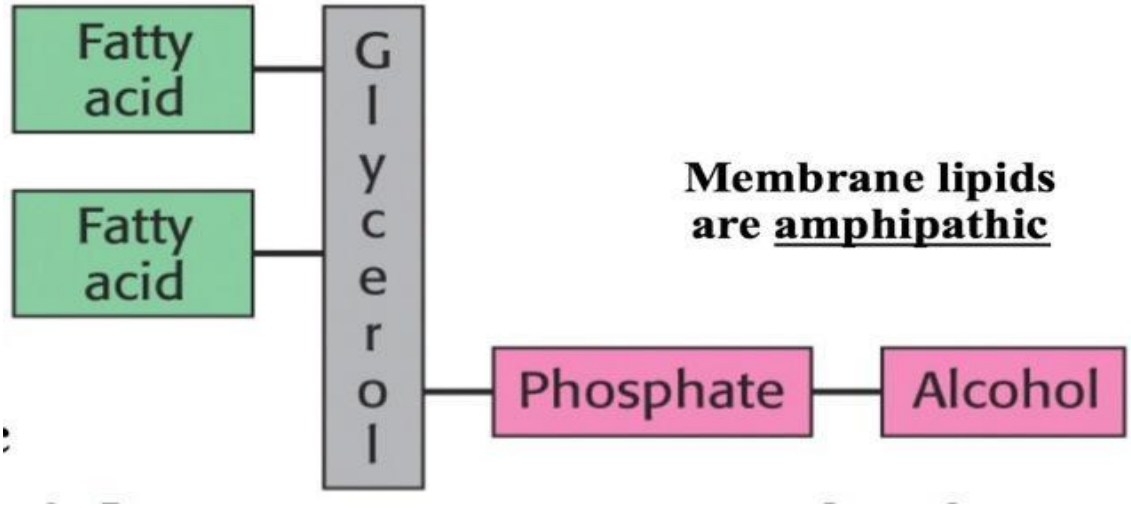
Biosynthesis of TAG (Triacylglycerol):



TAG : glycerol molecule esterified with 3 fatty acids.

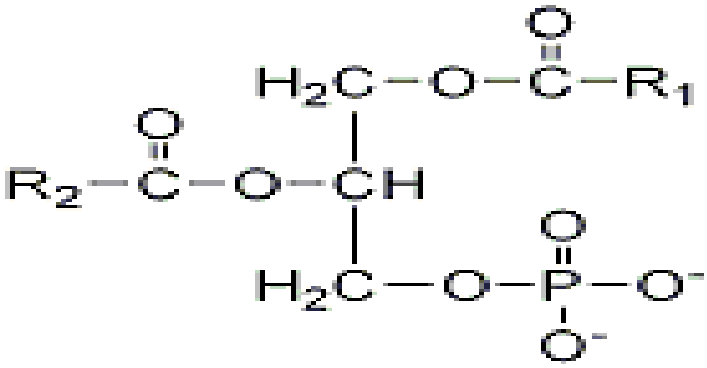
Note: we will also study **phosphoacylglycerol** which is (picture):



So it's similar to TAG but instead of the 3rd fatty acid, we have phosphate and alcohol. Note that phosphate can form 2 ester bonds: one with glycerol and one with the alcohol.

- TAG→energy storage. While phosphoacylglycerol→membrane components (membrane lipids).
- Since a part (glycerol + 2 fatty acids) is common between the two, we would expect similarities in biosynthesis of both molecules.

- The **common intermediate** between both TAG and Phosphoacylglycerol biosynthesis pathways is **Phosphatidic acid.**



Phosphatidic acid.

- **Phosphatidic acid:** glycerol esterified with 2 fatty acids and a phosphate group. If the phosphate is ionized (like in picture) then it is phosphatidate.
- Biosynthesis of Triacylglycerol

Requires

- Acyl~CoA (Active form of FATTY ACID, contains high-energy bond)
- Glycerol Phosphate

- Why do we need the activated fatty acid?

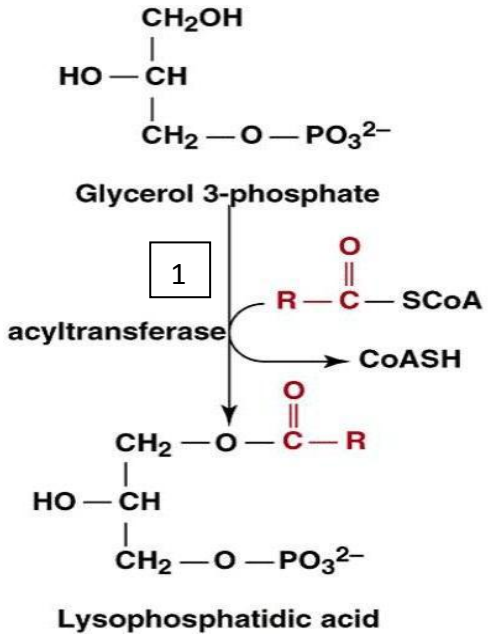
$$TAG + H_2O \Longrightarrow DAG + FA \quad (\Delta G - ve)$$

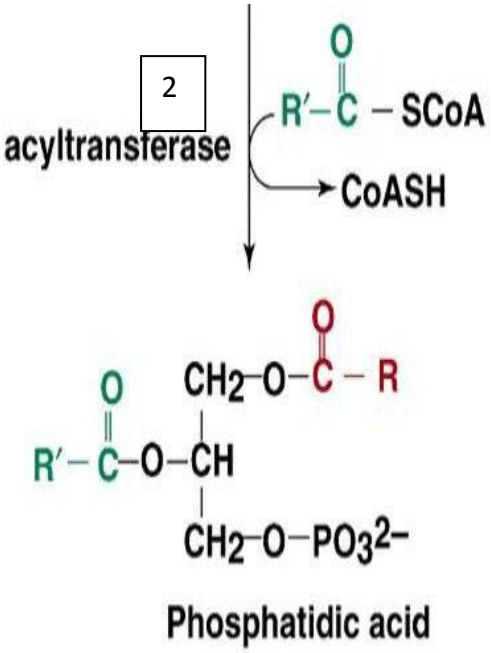
$$DAG + FA \Longrightarrow TAG + H_2O \quad (\Delta G + ve)$$

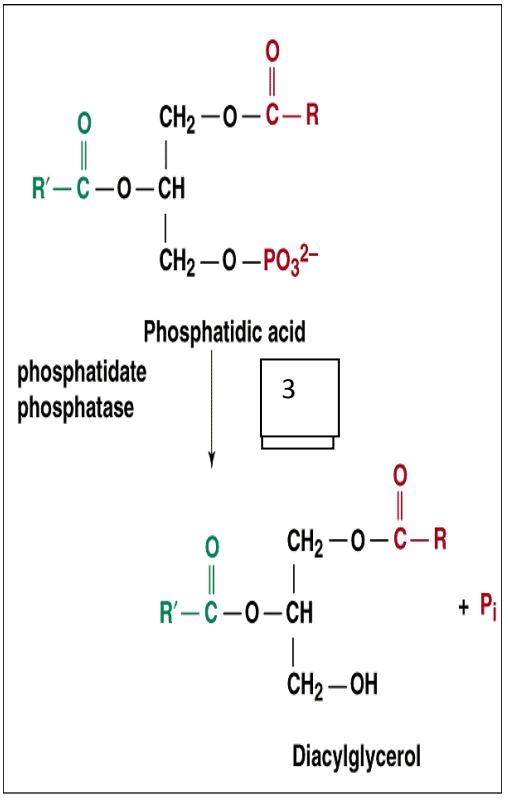
$$DAG + Acyl\sim CoA \Longrightarrow TAG \qquad (\Delta G - ve)$$

Regarding to the 1st rxn, it's a hydrolysis rxn, and hydrolysis reactions are ALWAYS exergonic reactions. The 2nd rxn MUST be endergonic, so it won't proceed on its own (unless you have massive amounts of DAG and FA and little amount of TAG) and this is hard to establish, so in order to make the rxn proceed, we need an energy source such as getting the FA from acyl-coa, because breaking of the high energy thioester bond releases significant amount of energy and makes ΔG negative.

➢ The steps:







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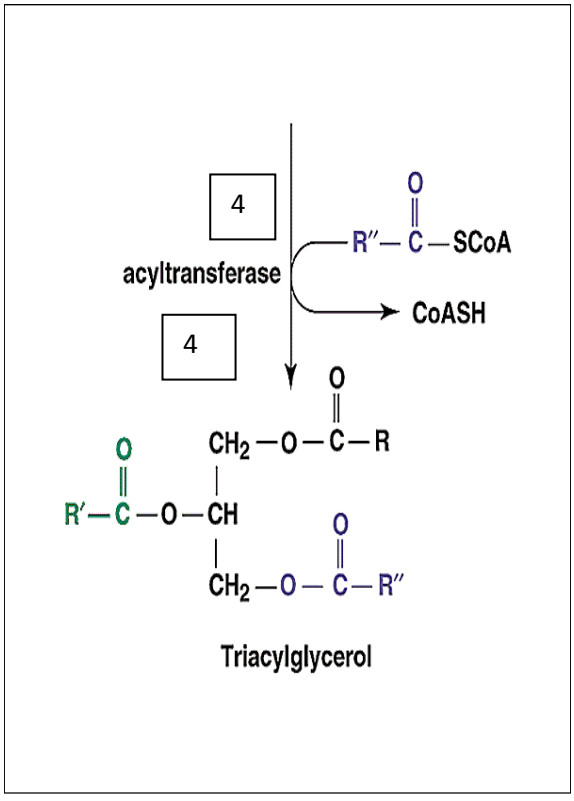

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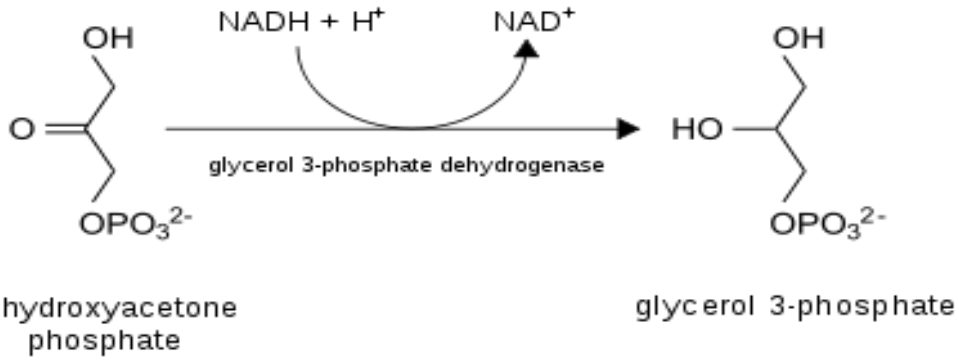
Production of Glycerol phosphate:

Glycerol + ATP ---→ Glycerol 3 Phosphate

Enz: Glycerol Kinase

The enzyme Glycerol kinase is Not present in Adipose tissue, so how does adipose tissue produce glycerol-3-phosphate?



By reduction of the Ketone group in DHAP into a hydroxyl group (Glycerol-3-phosphate).

- Most of TAG made in the body is made in the adipose tissue (for storage) and in the liver (in order to convert excess carbohydrates to fat, then transporting that fat to adipose tissue for storage).

- The adipose tissue can ONLY produces glycerol phosphate by glycerol-3-phosphate dehydrogenase (using DHAP), but the liver can utilize both DHAP and Glycerol (through glycerol kinase) to make it, but why?

  *If we study adipocytes, 90% of the adipocyte consists of TAG, if energy is needed; hormone sensitive lipase hydrolyses TAG into 3 fatty acids and glycerol. The fatty acids are carried to different tissues and glycerol is carried to the liver. While if synthesis of TAG is taking place, we need glycerol phosphate and Acyl-CoA.
  If (in adipocytes) glycerol can be converted to glycerol phosphate (the kinase is there),what will happen is that glycerol will get phosphorylated (1 ATP is used), and to make Acyl-CoA from a fatty acid we consume equivalent of 2 ATPs, so to make a TAG molecule, we need a glycerol phosphate and 3 Acyl-CoA molecules, which means we need 7 ATP molecules (1+3x2), so if the glycerol kinase is present in adipocytes, and we keep degrading and synthesizing TAGs, we're just achieving LOSS OF ATP and this is useless. So to prevent continuous synthesis and degradation of TAGs which will only result in loss of energy, glycerol kinase is NOT present in adipocytes.

- So how is glycerol phosphate made in adipocytes? It's made from glucose: Glucose enters the Adipocyte→Glycolysis→DHAP →glycerol phosphate. But how does glucose enter the adipocyte? By GLUT-4,

which is **insulin dependent. So** if insulin is available, we can make TAG, if insulin is not available→ no TAG synthesis.

- Note: some diabetic patients complain from weight loss without actively trying to lose weight. This occurs in diabetic patients whose pancreas don't secrete a lot of insulin→no glucose entry →noTAG synthesis, while degradation is active→ loss of weight in uncontrolled diabetes.

The End