Notes related to studying

- Prof. Faisal Al-Khateeb will give us **10 lectures** which will include **4 chapters** (*15, 16, 17 & 18*) with some omissions. He will **not** follow the textbook's order.
- The professor recommends referring to the textbook since it is "*… very good, it has lots of information but is summarized … the information is well-presented*".
- On a different occasion, he said that we have 3 studying resources (**Textbook, slides & sheets**) and **any two** of them will **suffice** for his material.
- This sheet will discuss **"Fatty Acid & Triacylglycerol Metabolism (Oxidation)** & **Utilization of Fatty Acids As A Source of Energy"** which is part of the "**Lipid Metabolism**" material.
- You can find this sheet's material in **Lippincott's Chapter 16**.

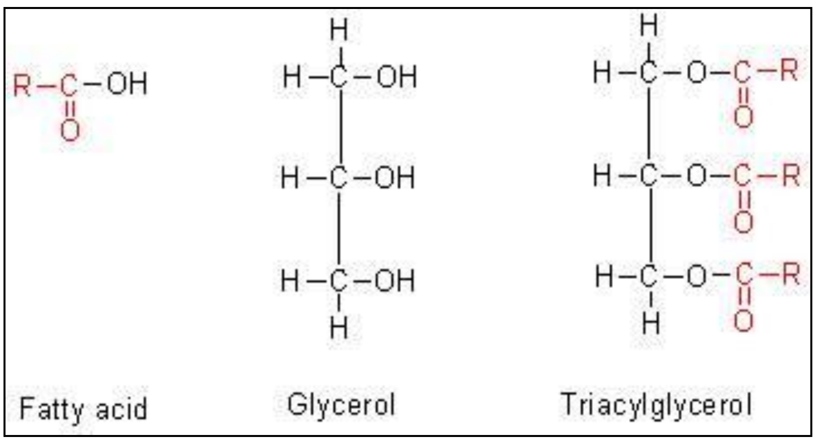


Figure 1

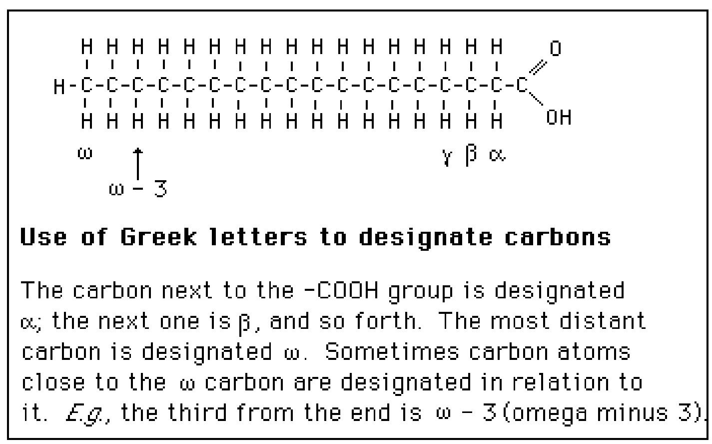


Figure 2

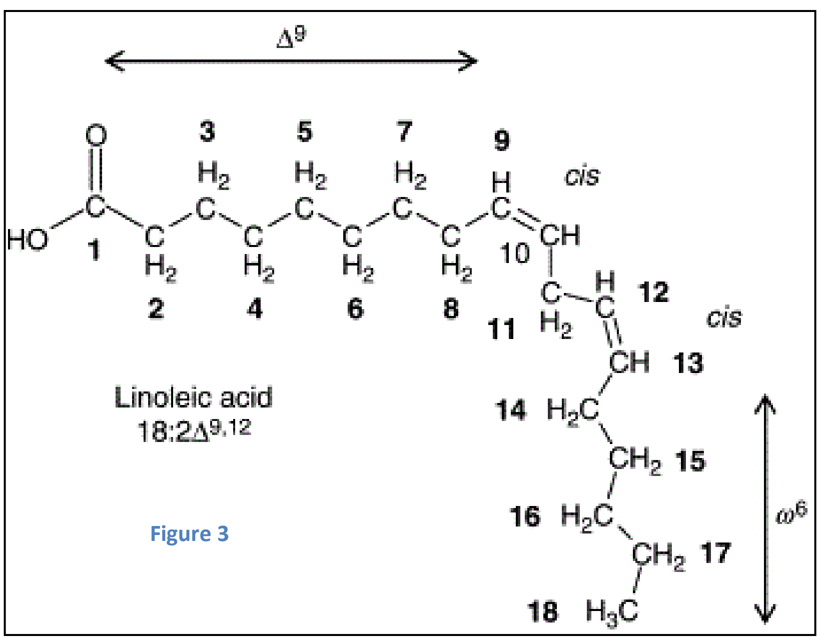


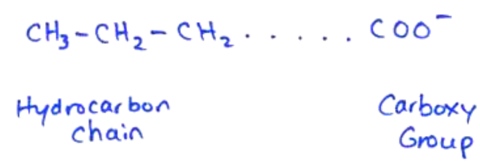
Figure 3

Nomenclature

- Triglyceride = Triacylglycerol = Fat
  - Tri → Refers to the 3 fatty acids in the structure.
  - Acyl → Fatty acid part which is involved in the ester bond.
  - Glycerol → Alcohol joined to the fatty acids.

Structure

- **Glycerol** (*3 carbon compound*) esterified to **3** fatty acids.[1]
- **Fatty Acid structure**
  - **Long hydrocarbon chain** (*Only C and H*)
    - Non-polar (*Hydrophobic*).
    - May or may not contain **double bonds**.
  - End is composed of a **carboxyl group** (*Hydrophilic*) so these fatty acids are **carboxylic acids**.
    - **Weak** acids.
      - pKa = 4.8
    - Makes them slightly more soluble than other compounds but generally, they are **insoluble in water** because of the long hydrocarbon chain.
    - All fatty acids are carboxylic acids but **not** vice versa.
  - Can be written as **$CH_3(CH_2)_n$ COO^-**, where n is the number of carbons.



Remember

- ✓ pKa is the **pH at which 50% of the molecules are ionized**.
- ✓ So if pH is **above** the pKa, the fatty acid's form will be **ionized** (*Will lose protons*).
- ✓ That means that fatty acids are **ionized at physiological pH** (*7.4*) because the pH is above their pKa.

Saturation of Hydrocarbon Chains in Fatty Acids

- Hydrocarbon chains in fatty acids **may or may not** contain double bonds.
  - **Saturated** fatty acids
    - **No** double bonds in the hydrocarbon chain structure.
  - **Unsaturated** fatty acids
    - **Monounsaturated** fatty acids
      - When there is **one** double bond in the chain's structure.
    - **Polyunsaturated** fatty acids
      - When there are **two or more** double bonds in the chain's structure
        - Double bonds are usually separated by a **Methylene group**.[2]

[1] Refer to figure 1 on page 1 (*2 with cover*).

Numbering Carbon Atoms in Long Hydrocarbon Chains

There are **two** major methods

1. By considering the **carbon in the carboxylic group** as the **first** carbon and counting from there.
2. By considering the first carbon **after** the carboxyl group as **α-carbon**, the one after it will be **β-carbon** and so on (*With Gamma (γ) and Delta (δ))*.
    a. **Regardless** of its number, the **last** carbon on the other end of the carboxyl group is **always Omega** (ω) because it's the last letter in the Greek alphabet.[3]
    b. This method can be used to determine the **location of double bonds** in the chain.

Determination of Double Bond Location [4]

- Using the **first** numbering method, you can notice that the first double bond is present at the carbon 9 while the second double bond is at carbon 12.
    - Double bonds are always **3 carbons apart**.
        - So if another double bond is to be introduced to this structure, it will be at carbon 15.
        - This also means that these are **non-conjugated** double bonds. [5]
    - Can be written as $\mathbf{18{:}\Delta 2^{9,12}}$ or 18:(9,12)
        - **18** is the **number of carbons** in the chain.
        - The number **after the Δ sign** is the **number of double bonds**.
        - The **superscripted** numbers represent the **locations of the double bonds**.
- Relying on the **second numbering** method, we can define the **ω carbon** and start numbering from it (*ω is the number one carbon*).
    - The first double bond will be present at carbon 6 so this fatty acid will be called **ω6**.
    - This method does **not** require you to write the location of other double bonds, **only the first one from the ω end matters**.
- **Connection between two methods**
    - **18** (*Number of carbons in the chain*) - **12** (*Location of last double bond in first method*) = **6** (*Location of first double bond in the second method*)

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[2] **Methylene** group is any part of a molecule that consists of **two hydrogen atoms** bound to a carbon atom, which is connected to the remainder of the molecule by a **double bond** (*Has the composition* $-CH_2-$).
[3] Refer to figure 2 on page 1 (*2 with cover*).
[4] Please refer to **slide 4** or **figure 3 on page 1** because they include the example (*Linoleic Acid*) used to explain this part.
[5] **Conjugated** double bonds are ones where single and double bonds **alternate** (*Single-Double-Single-Double*)

Fatty Acids of Physiological Importance [6] [7]

- From the table[8], you can notice that most of these fatty acids have an **even** number of carbon atoms.
    - Fatty acids with an odd number of carbons atoms are **very rare**.
- You can also notice that fatty acids are usually referred to by their **common name** rather than their IUPAC name.[9]
    - Common names were given to fatty acids based on the **natural sources of which they were first extracted or isolated**.

Fatty Acid	First Isolated in
Butyric	Butter
Capric	Butter or Goat (*Capric*) Milk
Palmitic/Palmitoleic	Palm Trees
Oleic	Olives
Linoleic/Linolenic	Linen[10] Seeds
Arachidonic	Peanuts[11]

"Whoa! *That* was a good one! Try it, Hobbs — just poke his brain right where my finger is."

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[6] Please refer to the table in slide 5.

[7] Formic Acid and Acetic Acid are both **commonly found** although the professor, personally, doesn't consider them to be fatty acids because **actual fatty acids are composed of 4 or more carbon**s so that makes **Butyric acid as the shortest (*Actual*) fatty acid**.

[8] The fatty acids with **arrows** pointed to them are the ones which structures (*Number of carbons and double bonds and their locations*) you **should know** because they will be repeated often, so memorize them.

[9] The professor mentioned the IUPAC names of some fatty acids but he said they weren't important so they weren't included in the sheet.

[10] Type of texture or fabric used to make tents (الكتان).

[11] Jimmy Carter, the 39th president of the United States, was a peanuts farmer.

Fat As An Energy Source

- Fats (triacylglycerol) are the **major energy reservoir** in the body which means that their main function is **energy storage**.
- Storage of energy as fat is **more efficient** than storing energy in the form of carbohydrates *(Glycogen for example, Glucose's polysaccharide in the liver*) for 2 reasons:
  - → **Fat is much more reduced** (*Contains less O*) than carbohydrates.
    - ▪ This makes the process of oxidation[12] (*Extracting energy*) more efficient (*Can be further oxidized than Glycogen*).
    - ▪ More energy can be obtained from oxidation of fat than carbohydrates (***9** kCal/gram vs.* ***4** kCal/gram*) so fat produces more than twice the amount of energy carbohydrates do.
  - → **Fats are more hydrophobic**
    - ▪ This makes their storage in cells (*Adipose tissue*) **more efficient** because **90%** of the fat cell is composed of TAG.
    - ▪ If fats were hydrophilic, they would **absorb water** (*Like carbohyrates*) which will **reduce the amount of fat in one cell** and increase the number of cells needed to store a certain amount of fat.
      - Every **gram of carbohydrates will store 2 grams of water** with it.[13]
  - → **Conclusion**
    - ▪ **10 kg** of fat are the average amount in a human body.
      - This amount will give **90,000 kCal/g** (*kilograms of fat * 1,000 g * 9 kCal*) or **90,000,000 Cal/g** of energy upon burning.
      - This will supply an average-weight human (*Daily energy requirement =* ***2000*** *kCal/day*) for **45 days** (*90,000 ÷ 2000*)
      - **Mass** of Carbohydrate required to produce the **equivalent** amount of energy to 10 kg of fat is: **90,000 kCal ÷ 4 kCal/g carbohydrate = 22,500 g = 22.5 kg of carbohydrates** (*Twice as much as fat*)
        - o But carbohydrates store water with them (*2 g H_2O → 1 g carbohydrates*) so **22.5 kg * 2 = 45 kg of water to be stored with 22.5 kg of carbohydrates.**
        - o 22.5 + 45 = **67.5 kg** (*Almost 7 times as much as the mass of fat needed for same amount of energy*).
    - ▪ This is the main reason **animals and humans store excess energy as fat** since they're always on the move and can't carry around that much weight while **plants** (*Rice, potatoes & grains*) **store excess energy as carbohydrates**.

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[12] The more a substance is reduced, the better oxidation reactions can go through.
[13] Indicates the great hydrophilic ability of carbohydrates.

	Fat	Carbohydrates
Dry Mass in Average Human	10 kg	22.5 kg
Wet Mass in Average Human	~ 10 kg	67.5 kg *((22.5 kg carbs * 2 kg H_2O) + 22.5 kg carbs)*
Energy Released	90,000 kCal *(10,000 g fat * 9 kCal/g fat)*	90,000 kCal *(22,500 g carbs [14] * 4 kCal/g carbs)*
Duration of Supply To The Body	45 days *(90,000 kCal ÷ 2000 kCal/day)*	45 days

Fatty Acids As Fuels

- Fatty acids are **not used only** during long fasts.
- They are the **preferred** fuel for **tissues**.
  - **Glucose** is the major **circulatory** fuel (*Used in blood*).
  - There are **very small** amounts of fatty acids in the blood under normal conditions.
- During a **12 hours** fast, the body consumes **540 kCal** (*90 g*) of fatty acids but at any given time, only **3KCal** (*0.3 g*) of fatty acids are **available in the blood**.
  - This means that the circulatory amount of fatty acids (0.3 g) is continuously going through **turnover** [15]**.**
  - Adipose tissue is **metabolically active** because it continuously produces fatty acids and releases them into the bloodstream to be used as source of energy.
- In comparison with Glucose, only **280 kCal** (*70 g*) are used during a **12 hours fast** and at any given time, **80 kCal** (*20 g*) are found in the bloodstream.
  - So Glucose is used to **a lesser extent** but is **widely available** which makes it an **ideal source** of energy for cells, especially those that cannot handle loss of energy for a long period such as the **brain and RBCs** (*Both cannot use fatty acids as source of energy*).

[14] Water doesn't contain any calories so it's not included in this equation.
[15] Constantly being used and replaced.

Fuel Type	Fatty Acids	Glucose
Energy Used During A 12 Hours Fast	540 kCal	280 kCal
Equivalent Mass	90 g	70 g
Amount of Energy in The Blood At Any Given Time	3 kCal	80 kCal
Equivalent Mass in The Blood At Any Given Time	0.3 g	20 g
Used Most in	Most Body Tissues	Brain & RBCs

Mobilization of Stored Fatty Acids

- Is the **First step** in using stored fatty acids
- Fat is usually stored in **Adipose tissue** for the use of **other** tissues (*Adipose tissue doesn't store fat for its own gain*) like the **liver and cardiac muscles**.
- When energy is needed, **adipocytes** are alerted by a **hormonal signal**.
  - This signal will cause **hydrolysis of TAG** in a reaction catalyzed by **Hormone-Sensitive-Lipase** (*Because this enzyme is only activated when specific hormones are present in the blood*).
  - There are 4 hormones which **activate** HSL enzymes:
    - High level of **Glucagon** in the blood indicates **hypoglycemia**[16].
    - High levels of **Epinephrine and Norepinephrine** indicate **increased need for energy** due to **stress** (*Same for **ACTH***[17]).

$$Triacylglycerol + 3\ H_2O \xrightarrow{Hormone\ Sensitive\ Lipase} 3\ Fatty\ Acids + Glycerol$$

- How do the aforementioned hormones (*Using Epinephrine as an example*) **stimulate mobilization** of fat?[18]
  - High levels of epinephrine mean more binding of Epinephrine to their **specific receptors** on the cell's membrane.
  - The attachment will activate **Adenylate Cyclase** (*or Adenylyl Cyclone*) which will subsequently cause conversion of **ATP to cAMP**.
  - **cAMP** (*Small soluble molecules*) will **diffuse** into the cytosol, and then it will bind to **and activate Protein Kinase A.**
  - Protein Kinase A **phosphrylates** HSL, converting it into the **active** form.
  - This will lead to the **hydrolysis** of **one** fatty acid converting **TAG to DAG** (*Diacylglycerol*) and
  - **Other enzymes** will remove the second and third fatty acids.

[16] Low levels of blood Glucose.
[17] Adrenocorticotropic hormone (*ACTH*), also known as corticotropin, is a polypeptide tropic hormone produced and secreted by the anterior pituitary gland. It is often produced in response to biological stress
[18] Please refer to slide 12.

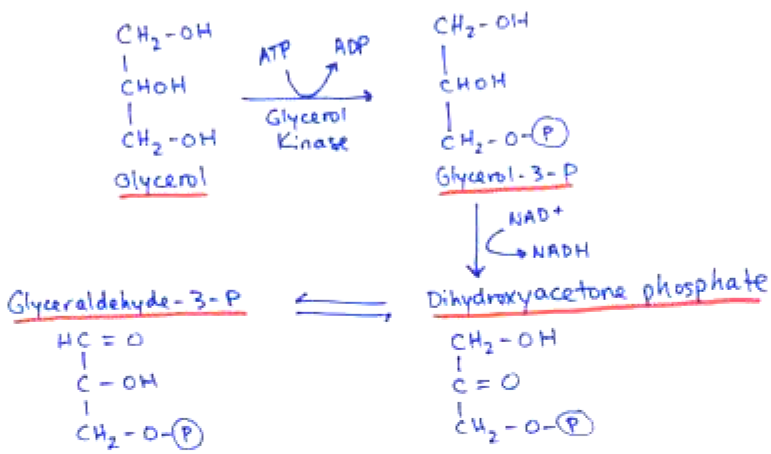
- Note that these events are similar to stimulating **glycogen degradation**.

> Take this as **a <u>general rule</u>**.
>
> When enzymes are **phosphorylated**, it leads to **sparing Glucose** (*Making it more available*). So when HSL are phosphorylated, this means more use of fatty acids instead of Glucose for energy.

<u>Fate of Glycerol</u>

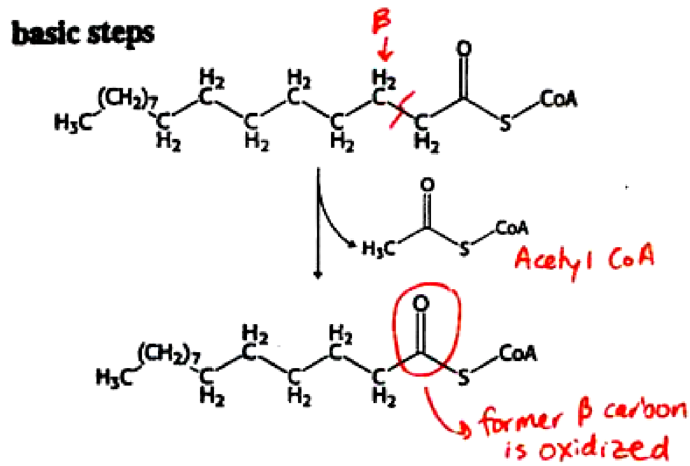
It will be released **into the bloodstream** and will reach the **liver**.

- First thing to occur to Glycerol in the liver is the **addition of a Phosphate group** (*From ATP*) to **carbon 3** by **Glycerol Kinase**.
- Glycerol 3-Phosphate's **middle OH is oxidized** and the **Hs (on middle C) are transferred to NAD^+** in order to **produce NADH**. This also produces **Dihydroxyacetone Phosphate**.
    - DHAP is an **intermediate** in glycolysis and gluconeogenesis.
    - In this case, DHAP goes to **gluconeogenesis** (*Predominant pathway*) because we need more Glucose in this specific body state.
- Despite the fact that glycerol's percentage in TAG is **less than fatty acids** (fatty acids have 16-18 C each, Glycerol only 3), it is the **only** part of TAG which **participates in producing Glucose**.



    - Fatty acids **cannot** be converted into Glucose so they will be used as source of energy.

<u>**Fate of Fatty Acids**</u>



- They will be transferred through the bloodstream to muscle, cardiac muscle, liver, etc.
    - Since they are **insoluble**, they will be **bound to Albumin** as to **prevent precipitation** in blood vessels.
- **Degradation of Fatty Acids**
    - Happens by **oxidation of β-carbon** (*Carbon 3*).
        - Called, obviously, **β-oxidation**.
        - CH_2 becomes **C=O** (*Ketone group*).
        - Occurs in the **mitochondrial matrix**.

<u>**Overview of β-oxidation**</u> [19]

→ First step is to **activate the FA** by **linking it with CoA**.

→ After **3** steps (*Reactions*), the **β-carbon is oxidized** (*Turned into a keto-acid*).

→ Next step is **cleavage of carbons 1 and 2** with the new molecule of CoA which will give us **Fatty Acyl CoA** (*Similar to the one we started with but it's **2 carbons less***) **and Acetyl CoA** (*Products of β-oxidation*).

→ Next step is repeating the aforementioned steps again and again (*Form cycles*) until the fatty acid is **completely oxidized to Acetyl CoA**.
- Even if the oxidation is repeated again and again, the activation of fatty acids by linking them with CoA, is done only **once**.

<u>**Question :**</u> Assume we started oxidizing a fatty acid with 16 carbons, how many cycles of β-oxidation do we need to completely convert it to a 2 carbons compound?

<u>**Answer :**</u> 7 cycles.

<u>**Explanation :**</u> Note that upon completion of turn 7, the four carbon fragment is split into 2 acetyl CoA groups. An eighth turn of the cycle is unnecessary since the eighth and last acetyl CoA has already been made. As a general principle, the number of fatty acid spiral turns is always one less than the number of acetyl CoA groups formed. In this example, 8 - 1 = 7 turns of the fatty acid spiral.

[19] Please refer to slide 15 (*Carbons 2 and 3 are colored in green*)

Activation of Fatty Acids

It is linking the fatty acid to CoA which forms a **RCO~SCoA** (*Thioester bond*).

- → The symbol "~" represents the **high energy** in this bond (*When hydrolyzed, releases energy similar to the hydrolysis of ATP*).
- → **Fatty acid + ATP + HS-CoA ↔ FAcyl-CoA + AMP + PPi** (*Pyrophosphate*).
  - o This reaction is **reversible** because one high energy bond is being formed (*Thioester bond*) and another high energy bond is being broken (*Between phosphate 1 and phosphate 2 of ATP*) so ΔG for this reaction is **close to zero** (*favoring reversibility*).
  - o This reaction is catalyzed by **Thiokinase** (*Acyl-CoA Synthetase*[20])
- → How can we make the overall reaction **irreversible**?[21]
  1. Continuously **removing** the products of the reaction in order to make it **favor the forward** direction.
  2. This can happen by cleaving (*Hydrolyzing*) **PPi into 2 Pi**.
     - ▪ **Very rapid** irreversible reaction.
     - ▪ Catalyzed by **Pyrophosphatase**.
- → How can we convert **AMP into ATP**?
  1. **AMP + ATP → ADP +ADP**
  2. Add this equation to the activation of fatty acids equation.
  3. Result : **Fatty Acid + CoA + 2 ATP → Fatty Acid-CoA + 2 ADP**
     - ▪ Activation of fatty acids costs **2 ATP** (*One is used directly while the other is used indirectly to recharge/reactivate AMP*)
     - ▪ Also catalyzed by **Thiokinase**.
  4. ATP conversion to AMP and 2 Pi is equivalent to hydrolysis of 2 ATP to ADP. If one of the ADP molecules formed during activation is converted to AMP, this would be equivalent using 2 high energy bonds (since ATP would have been first converted to ADP which was then again converted to AMP).

Question : How many ATP molecules will be used by Thiokinase or Fatty Acid Synthetase?

Answer : 1 ATP

Thiokinase

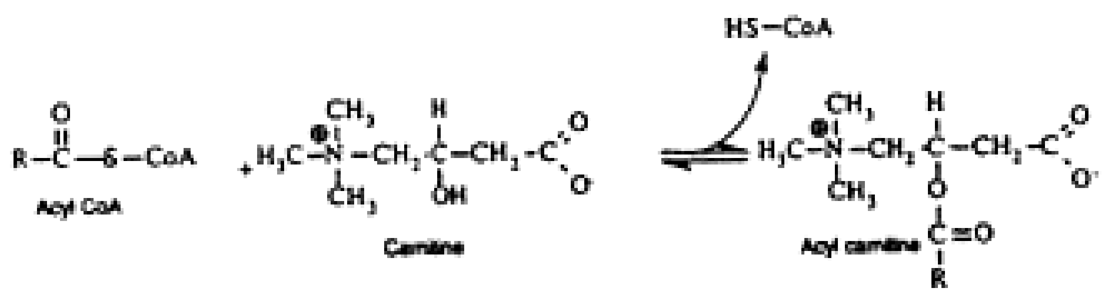
- → Main enzyme in activation of fatty acids (*Specific for chain length*)
- → Location
  - o **Long** Chain Fatty Acids (*14+ carbons*) → **Outer Mitochondrial Membrane**
  - o **Short** (*4 carbons*) and **Medium** (*8 - 10 carbons*) → **Mitochondrial Matrix** (*Can be activated directly in the matrix*)

[20] The difference between synthase and synthetase: is that ATP is needed in synthetase

[21] Please refer to slide 16

Transport of Long Chain Acyl CoA Across Inner Mitochondrial Membrane

- **Inner** mitochondrial membrane is **impermeable** to Acyl CoA and H^+ (*Smallest molecule*).
- A carrier system is **required** and this system is known as **Carnitine Shuttle**. It consists of:
  - Carrier molecule
  - 2 enzymes
  - Membrane transport protein
- The Carnitine Shuttle is required for the entrance of the Acyl group into mitochondria.
- Fatty Acyl CoA is produced in the OMM, it can enter the IMM without a shuttle
- First step: **Carnitine Palmitoyl-Transferase I** *(CPT)*[22] transfers a fatty acyl group from Fatty acyl CoA to Carnitine producing **Acyl Carnitine**.
- **Translocase** helps move the Acyl Carnitine from the intermembrane space into the **matrix**.
- The acyl group is then **transferred back** to CoA to produce **Fatty Acyl CoA** by **CPT II**. Carnitine then goes back.
- Conclusion :
  - **Two** enzymes are needed.
  - It is called a **shuttle**[23] because it transports acyl CoA into the mitochondria, into the matrix, and back into the intermembrane space (*Goes back and forth*).



"We can easily forgive a child for being afraid of the dark, but the tragedy is when men are afraid of the light"

Awkward moment when the footnotes can comprise a sheet of their own.

Great luck.

[22] Also known as Carnitine Acyltransferase I.
[23] مكوك