Fatty Acid and Triacylglycerol Metabolism

Fat, oil, triacylglycerols, triglycerides all refer to the same thing. Triacylglycerol is based on its chemical composition; it is a glycerol esterified with 3 fatty acids, which is why it is called Triacylglycerol. Its architecture is glycerol with 3 carbons, with 3 hydroxyl groups. It is particularly made of different fatty acids, so called triacylglycerol. Also called fat if in food. A triglyceride (TG, triacylglycerol, TAG, or triacylglyceride) is an ester derived from glycerol and three fatty acids. There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so.

Triglycerides are formed by combining glycerol with three molecules of fatty acid. Alcohols have a hydroxyl (HO-) group. Organic acids have a carboxyl (-COOH) group. Alcohols and organic acids join to form esters. The glycerol molecule has three hydroxyl (HO-) groups. Each fatty acid has a carboxyl group (-COOH). In triglycerides, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds.

Fatty Acid Structures, the most important of them:

Fatty acids have general formulae: a long Hydrocarbon chain that ends with a carboxyl COO- group with only 2 oxygen's, so it is a largely reduced chain with high numbers of hydrogen's. So is mostly made of a hydrogen-carbon long chain.

The pka of the carboxyl COO- group is 4.8, and if the PH is higher than the pka, the acid is in an unprotonated/ ionized form i.e. will lose protons if PH>Pka and exist as an anion. E.g. If the PH is 7, which is around our physiological PH, the fatty acid will exist in the ionized COO- form. What would you expect to find if PH<Pka? More of the non-ionized COOH form.

This can be written as CH3(CH2)n COO- , where n is the number of carbons.
In a long hydrocarbon chain, designation of the different carbons can be done in two ways:

- Either start counting from COO- carbon, considering it as carbon #1.
- Or by counting them in greek letters:
  - α carbon as being carbon #2 after the COO-, β carbon as carbon #3, γ carbon as carbon #4, and δ (delta) carbon as carbon #5.

![Carbon numbering diagram](image)

The last Carbon at the other end of the COO- group, regardless of its number, is the ω (omega) carbon. ω represents the last letter of the greek alphabet.

This numbering can be used in the determination of the c=c double bond location.

N.B:
- γ and δ are less frequently used in numbering, unlike the rest.

The Hydrocarbon chain may or may not have double bonds.

Fatty acids with a single (c=c) double bond are called mono-unsaturated fatty acids.
Fatty acids with ≥ 2 (c=c) in different locations, are called poly-unsaturated fatty acids.

**To differentiate double bond location:**

- **Using the first way:**

  Linoleic acid, is an 18 Carbon poly-unsaturated fatty acid (specifically with 2 c=c bonds), with c=c location at c#9 and c#12 (with respect to COOH).

  Expressing Linoleic acid in short hand notation would be **18:2Δ9,12**

  N.B Remember to give the carbon closest to the COOH the priority in numbering the c=c.
Using the second way in differentiating double bond location:

Try counting from the omega (ω) carbon. (the furthest away carbon from COOH). So Linoleic acid can also be called Omega 6.

N.B We do not call Linoleic acid omega 6 and 9, but rather Omega 6 alone because if the location of the first double bond is known, and the number of double bonds is also known, a simple addition of 3 will indicate the location of the next c=c bond.

E.g. Linolenic acid is a poly-unsaturated (3c=c’s) 18C fatty acid, with the first c=c at ω 3 which is C#15, so the rest of the c=c locations will be at C#(15-3) and C#(12-3) i.e. Linolenic acid is 18:3Δ9,12,15

N.B The polyunsaturated fatty acid system is not a conjugating one. Instead, is separated by methylene CH2 groups and as mentioned earlier, substract 3 to locate the next c=c location from the ω carbon. (Non-conjugating double bonds of fatty acids which are 3 carbons apart)
Some Fatty Acids of Physiological Importance:

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Structure</th>
<th>Location of c=c if present</th>
<th>IUPAC name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric acid</td>
<td>4:0</td>
<td></td>
<td>butanoic acid</td>
</tr>
<tr>
<td>Capric acid</td>
<td>10:0</td>
<td></td>
<td>Decanoic acid</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>16:0</td>
<td></td>
<td>hexadecanoic acid</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>16:1 Δ9</td>
<td></td>
<td>(9Z)-9-Hexadecenoic acid</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>18:0 Δ9</td>
<td></td>
<td>octadecanoic acid</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>18:1 Δ9</td>
<td></td>
<td>(Z)-octadec-9-enoic acid</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18:2 Δ9,12</td>
<td></td>
<td>(9Z,12Z)-9,12-Octadecadienoic acid</td>
</tr>
<tr>
<td>α-Linolenic acid</td>
<td>18:3 Δ9,12,15</td>
<td></td>
<td>(9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>20:4 Δ5,8,11,14</td>
<td></td>
<td>(5Z,8Z,11Z,14Z)-5,8,11,14-Icosatetraenoic acid</td>
</tr>
</tbody>
</table>

These fatty acids are known by their Common names. These names have been given depending on the rich source from which they have been isolated from.

Butyric acid abundant in the fat of butter, Palmitic/Palmitoleic acid from Palm oil, Stearic acid from a form of beef/animal fat, Linoleic/Linolenic acid from cell membrane lipids of vegetable oils (Lin seeds, sunflower, and corn oils). Arachidonic acid from peanuts.

Remember fat, oil, triacylglycerols, triglycerides all refer to the same thing.

✓ Fats are the Major Energy Storage Reserve in the body.

About 90% of the volume of adipocytes is fat.
There are 2 reasons for which it is more efficient to store energy in the form of fats than in the form of carbohydrates.

1. The fat is more reduced, i.e. with less amount of oxygen, and since oxidation is needed for energy use, the more the substance is reduced, the more the oxidation processes it can go through, making fat an ideal energy source.

   9 kcal per gram of fat is produced compared with only 4 kcal per gram carbohydrate.

<table>
<thead>
<tr>
<th>Upon combustion:</th>
<th>Energy released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per gram Fat</td>
<td>9 kcal</td>
</tr>
<tr>
<td>Per gram Carbohydrate</td>
<td>4 kcal</td>
</tr>
</tbody>
</table>

   Fat combustion almost produces twice as much energy than that of carbohydrates
   9 kcal of fat = 2*(4 kcal) of carbohydrate

2. Fat is Hydrophobic, and can be stored without water in adipose tissue occupying 90% of the adipocytes volume.

   If hydrophilic carbohydrates, like glycogen or cellulose, were to be stored, they will absorb water and become hydrated.

   For every gram carbohydrate, 2 grams of water will be absorbed, indicating the great hydrophilic ability of carbohydrates.

   **1g carbohydrate: 2g H₂O**

   Since fat is hydrophobic (w/o H₂O), more caloric fat can be stored with the least mass possible, since non-caloric water is not associated with it.

   An average adult has 10kg of fat, which is about

   10,000g * 9 kcal = 90,000 kcal of energy/g or 9,000,000 cal/g in 10kg fat.

   Average energy needed for a person per day is about 2,000 kcal.

   So the **10kg Fat** will supply energy up to 90,000 (kcal/10kg) ÷2000 (kcal/day) = **45 days** in an average weighted person
The mass of Carbohydrate required to produce the equivalent amount of 10kg fat energy is 90,000(kcal) ÷4 (kcal/g carbohydrate) =22,500g carbohydrate= 22.5kg of carbohydrate

But since carbohydrates are hydrophilic, and upon storage need 2g H₂O per gram carbohydrate, the 22.5kg anhydrous carbohydrate is accompanied with (22.5*2)= 45kg H₂O
So the total mass is 22.5kg carbohydrate + 45kg H₂O = 67.5 kg

If the same amount of energy in 10kg fat is stored as hydrated carbohydrate, 67.5kg of mass would be required which is too large for our body weight to carry around.
People would weigh almost 7 times as much! [67.5kg hydrated carbohydrate: 10kg fat]
Regardless of the fact that those valuable carbohydrates, may be used in other metabolic pathways too.

Energy in 10kg Fat ≈ Energy in 22.5kg anhydrous carbohydrate ≈ Energy in 67.5kg hydrated carbohydrate, since water is non-caloric in value.

Animals usually move and so they store fat, while plant seeds store carbohydrate.

Fatty acids as fuels

Fatty acids are the major fuel of energy used by tissues but glucose is the major circulating fuel.

The blood glucose level is much higher than the amount of blood fatty acids. A 12hr fast will consume almost 90g (540kcal) of fatty acids, and at any one time there is always <1g (3kcal) of fat in the plasma, so fatty acids will enter the plasma continuously because they are used continuously. However, if glucose were the fuel in a 12hr fast, almost 70g (280kcal) would be needed, and at any one time, the amount of glucose in the fluid is almost 20g (80kcal).

A gram of glucose gives 4 kcal, and remember that fatty acid energy is dependent on the type of fatty acid.

<table>
<thead>
<tr>
<th>Fuel Type</th>
<th>Energy used in a 12hr fast</th>
<th>Equivalent Mass</th>
<th>Energy in fluids</th>
<th>Equivalent Mass in fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acids</td>
<td>540 kcal</td>
<td>90g</td>
<td>3kcal</td>
<td>&lt;1g</td>
</tr>
<tr>
<td>Glucose</td>
<td>280 kcal</td>
<td>70g</td>
<td>80kcal</td>
<td>20g</td>
</tr>
</tbody>
</table>

This table indicates that fatty acids have a greater turnover number than that of glucose. Fatty acids are more frequently substituted, are continuously changed and remain in the plasma for a relatively short period of time before being replaced.
Mobilization of Stored Fats

Fat is stored in specialized adipose tissue, and its energy is used by other types of tissues (liver, muscles...etc). When fat energy in the adipose tissue is needed; adipocytes are informed by a must hormonal signal which results in triggering hydrolysis of triacylglycerides for its transport in the plasma.

\[
\text{Triacylglyceride} + 3H_2O \rightarrow 3 \text{ Fatty acids} + \text{Glycerol}
\]

This hydrolysis not only involves breaking the 3 ester bonds in the triglyceride by 3 molecules of water, but also only occurs if an enzyme called a Hormone Sensitive Lipase (HSL) is active. As its name suggests; this enzyme is a lipase that, with the presence of water, can break the triglyceride ester bonds and can also be activated, or is sensitive to specifically 4 hormones. The hormone sensitivity is characteristic to this type of lipase.

The 4 hormones that can activate HSL enzymes are:

- **Glucagon**: a high amount of glucagon indicates a low concentration of blood glucose, for example, in a fasting state, and so fatty acids are used instead of glucose in hypoglycemic situations. The formation of glucose from fatty acids is a low pay work.
- **ACTH** (Adrenocorticotropic hormone)
- **EP**
- **NE**: Both EP and NE are secreted during stressful situations, which is usually needed to provide energy for muscle contraction by the action of HSL's
The mode of action of these hormones is by binding to specific receptors.

This mobilization of stored Glycogen in Glycogenolysis is also Glucagon-dependant, and is similar to that in the liver. High glucagon means fat should be used as the source of energy.

Remember that phosphorelation is a way to spare glucose and occurs when glucose is preserved.
Glycerol from triacylglycerol hydrolysis will be transported into the liver.

Once in the liver, ATP is used to phosphorolate C#3 of glycerol with the help of glycerol kinase, forming glycerol-3-phosphate.

Then, Glycerol-3-phosphate can be oxidized at C#2 to form dihydroxyacetone phosphate (DHAP).
DHAP is an intermediate of glycolysis and gluconeogenesis. But more of the DHAP will act as an intermediate of Gluconeogenesis, since phosphorelation is done to preserve glucose when glucose levels are low, and this gluconeogenesis is done in a fasting liver.

N.B
Glycerol is 3 carbon long and can be converted to glucose, however, fatty acids cannot be converted to glucose.

Fatty acids are the other product (besides glycerol) of the triglyceride hydrolysis.

Fatty acids are released by the adipocyte, and are carried by Albumin simply because fatty acids are insoluble in water if alone, and albumin helps carry it. Fatty Acids are transported to tissues being itself bound to albumin.

Oxidation of Fatty acids is through β oxidation:

Fatty acids are degraded by oxidation at their β carbon (C #3) followed by cleavage of two carbon units.

-CH₂- → -C-

This is the Carbon #3 of the fatty acid (the β carbon). It is oxidized to a ketone (C=O) group.

Oxidation of Fatty acids is through β oxidation:

Fatty acids are transported into the cell and carried into the mitochondria. β oxidation occurs in a series of steps in the mitochondrial matrix and is repeated again and again.

- The first step of utilizing/activating fatty acids for β oxidation, is linking the fatty acid with CoA.
- then in 3 steps, the β carbon is oxidized to a ketone group, then,
- degradation of the fatty acid occurs between C#2 and C#3(β carbon), forming 2 carbons in the form of Acetyl CoA and the rest as fatty acyl CoA.
This Fatty acyl CoA product of β oxidation is 2 Carbons less than the original fatty acid. (Cleavage of 2 Carbon units)

N.B  
Even if the oxidation is repeated again and again, the activation of fatty acids by linking them with CoA, is done only ONCE.

**Looking separately at the Activation of Fatty acids:**

The first step in activation, as mentioned earlier, is the linking of fatty acids with CoA. This forms a RCO~SCoA (Thioester bond), which is a highly energetic bond.

\[
FA + HSCoA + ATP \rightleftharpoons FA~CoA + AMP + PP_i
\]

This reaction is reversible, because from an energy point of view, one high energy bond is formed (Thioester bond), and one high energy bond is broken (Between phosphate group 1 and phosphate group 2 of ATP). \(\Delta G\) for this reaction is close to zero favouring reversibility.
To make the first reaction in fatty acid oxidation irreversible, continuously remove the product of the reaction to favour the forward reaction. Rapid hydrolysis of inorganic pyrophosphate (PPI) as soon as it is formed, produces 2 inorganic phosphates (2Pi), with the help of Pyrophosphatase.

- Overall, 2 high energy bonds are cleaved; one from ATP and another from PPI, to form 1 high energy bond in Fattyacyl~CoA (thioester bond).

The produced AMP accepts a phosphate group from ATP, forming 2 ADP molecules. The ATP transfer of its Pi group to AMP is used to regenerate ATP from the 2 ADP molecules.

- Overall, the formation of one Fattyacyl~CoA, consumes 2ATP.

**ATP conversion to AMP and 2 (Pi), is equivalent to hydrolysis of 2ATP to 2ADP.**

If one of the ADP molecules formed during activation is converted to AMP, this would be equivalent to using 2 high energy bonds.

The number of ATPs needed for activation would be 2 ATPs. However, the two ATPs have not directly participated in the reaction.

**The second step in fatty acid activation is the Activation of ATP**

(Its conversion to AMP), is done by the enzyme thiokinase (Acyl CoA Synthetase)

N.B

The difference between the synthase and synthetase, is that if ATP is needed, or is part of the reaction, then it is called synthetase. Unlike synthase which does not require ATP in the reaction.
The Activation of ATP happens in the:

- **Outer mitochondrial membrane for long chain fatty acids.**
- **Mitochondrial matrix for short and medium chain fatty acids.** (these chains can enter into the matrix)

[ Short chain=4C long, Medium chain= (8-10)C long, Long chain= ≥14C long ]

Remember that the β Oxidation of fatty acids happens in the mitochondrial matrix.

**Transport of long chain Acyl CoA across inner mitochondrial membrane**

**Inner mitochondrial membrane is impermeable to Acyl CoA** and so its transport into the mitochondrial matrix requires a carrier.
The *inner* mitochondrial membrane allows protons(H+) inside to allow acyl CoA inside.

Carrier system is required (Carnitine Shuttle) **that consists of**:

- A Carrier molecule → Carnitine
- Two enzymes → Carnitine palmitoyl-transferase I and II
- Membrane transport protein → Translocase

Carnitine palmitoyl-transferase I transfers a fatty acyl group from Fatty acyl CoA to carnitine. (the fatty CoA part of the fatty acyl CoA is usually palmitoyl CoA.)
The newly acylated carnitine in the intermembrane space, can enter the matrix with the help of a translocase. Inside the matrix, the same reaction happens but in the opposite direction. **Carnitine palmitoyl-transferase II** transfers the fatty acyl group back to CoA, and free carnitine, with the help of the same translocase, can be transported back into the intermembrane space.

As if Carnitine acted as a method of transport for fatty acyl CoA into the mitochondria, into the matrix, and back into the intermembrane space. This explains it being named a **Carnitine Shuttle**.

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