

## Fatty Acid Biosynthesis

- Synthesis takes place in the cytosol
- Intermediates covalently linked to acyl carrier protein
- Activation of each acetyl CoA.
- $\text{acetyl CoA} + \text{CO}_2 \rightarrow \text{Malonyl CoA}$
- Four-step repeating cycle, extension by 2-carbons / cycle
  - Condensation
  - Reduction
  - Dehydration
  - reduction

## Fatty acid synthesis

- The enzymes of fatty acid synthesis are packaged together in a complex called as fatty acid synthase (FAS).
- The product of FAS action is palmitic acid. (16:0).
- Modifications of this primary FA leads to other longer (and shorter) FA and unsaturated FA.
- The fatty acid molecule is synthesized 2 carbons at a time
- FA synthesis begins from the methyl end and proceeds toward the carboxylic acid end. Thus, C16 and C15 are added first and C2 and C1 are added last.
- C15 and C16 are derived directly from acetylCoA. For further step-wise 2-carbon extensions, acetylCoA is first activated to malonyl CoA, a 3-carbon compound, by the addition of a  $\text{CO}_2$ .

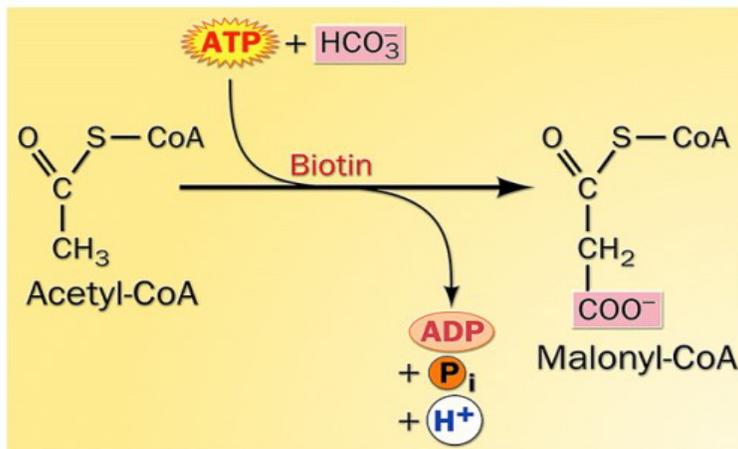
### Citrate Shuttle

- FAs are synthesized in the cytoplasm from acetylCoA
- AcetylCoA generated from pyruvate by the action of PDH and by  $\beta$ -oxidation of fatty acids is in the mitochondria.
- For fatty acid biosynthesis, acetylCoA has to be transported from the mitochondria to the cytoplasm. This is done via a shuttle system called the **Citrate Shuttle**.
- AcetylCoA reacts with oxaloacetate to give citrate. A **tricarboxylate translocase** transports citrate from mitochondria to cytosol.
- In the cytosol, citrate is cleaved back to oxaloacetate and acetylCoA. This reaction is catalyzed by ATP-citrate lyase and requires the hydrolysis of one molecule of ATP.

### Citrate Shuttle (regeneration of pyruvate)

- Oxaloacetate is converted back to pyruvate for re-entry into mitochondria
- Step 1. Oxaloacetate + NADH + H<sup>+</sup> → malate + NAD<sup>+</sup>. Reverse of the TA cycle reaction. Catalyzed by cytosolic malate dehydrogenase.
- Step 2. Malate + NADP<sup>+</sup> → pyruvate + CO<sub>2</sub> + NADPH. Catalyzed by malic enzyme
- Pyruvate translocase transports pyruvate into mitochondria.
- Pyruvate is converted to oxaloacetate by pyruvate carboxylase with coupled hydrolysis of one ATP. Pyruvate + ATP + CO<sub>2</sub> + H<sub>2</sub>O → oxaloacetate + ADP + Pi (reaction of gluconeogenesis)
- Net Reaction: NADP<sup>+</sup> + NADH + H<sup>+</sup> + ATP + H<sub>2</sub>O → NADPH + NAD<sup>+</sup> + ADP + Pi
- Thus, transport of acetylCoA to cytosol requires expense of one ATP and conversion of one NADH to NADPH.

### Reaction catalyzed by Acetyl CoA Carboxylase



Activation of acetate : Acetyl-CoA to malonyl CoA

### MalonylCoA

- Malonyl CoA is synthesized by the action of acetylCoA carboxylase. Biotin is a required cofactor.
- $\text{CH}_3\text{COSCoA} + \text{CO}_2 + \text{ATP} \rightarrow \text{OOC-CH}_2\text{-CO-SCoA} + \text{ADP} + \text{P}_i$  (enzyme: acetylCoA carboxylase)
- This is an irreversible reaction. AcetylCoA carboxylation is a rate-limiting step of FA biosynthesis.
- AcetylCoA carboxylase is under allosteric regulation. Citrate is a positive effector and palmitoyl CoA is a negative effector.

### Fatty Acid Synthase (FAS)

- FAS is a polypeptide chain with multiple domains, each with distinct enzyme activities required for fatty acid biosynthesis.
- ACP: Recall that CoA is used as an activator for  $\beta$ -oxidation. For fatty acid biosynthesis, the activator is a protein called the acyl carrier protein (ACP). It is part of the FAS complex. The acyl groups get anchored to the CoA group of ACP by a thioester linkage
- Condensing enzyme/ $\beta$ -ketoacyl synthase (K-SH). Also part of FAS, CE has a cysteine SH that participates in thioester linkage with the carboxylate group of the fatty acid.
- During FA biosynthesis, the growing FA chain alternates between K-SH and ACP-SH

### Step-wise reactions

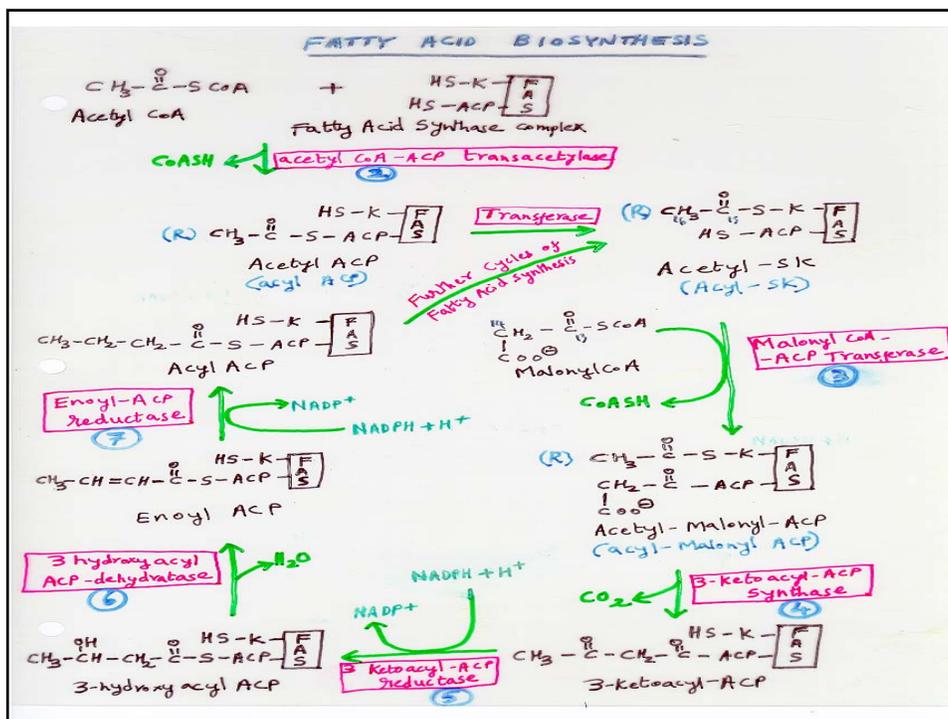
1. The **acetyl group gets transferred from CoA to ACP** by **acetyl CoA-ACP transacylase**.
2. The **acetyl (acyl) group next gets transferred to the K arm** of FAS complex.
3. Next, the **malonyl group gets transferred from CoA to ACP** by **malonyl CoA ACP transacylase**. This results in both arms of FAS occupied forming acyl-malonyl-ACP.
4. The COO group of malonyl ACP is **removed as CO<sub>2</sub>**, the **acetyl group (C16 and C15) gets transferred to the alpha carbon of malonyl ACP**. This results in **3-keto acyl ACP**.

### Reactions of FA biosynthesis (continued)

- The 3-keto group is converted to a CH<sub>2</sub> by a series of reactions reverse to FA β-oxidation. Namely,
  1. reduction to hydroxyl group. Enz: 3-keto acyl ACP reductase
  2. dehydration to form a 2,3 double bond and Enz: 3-hydroxy acyl ACP dehydratase
  3. a second reduction to remove the double bond. Enz: Enoyl ACP reductase
- Both reduction reactions require the reduced **cofactor NADPH**. This is **generated by the hexose monophosphate pathway** of phosphogluconate pathway and during the citrate shuttle

### Repeat cycles for chain elongation

- The result of the first cycle of fatty acid biosynthesis is a four carbon chain associated to the ACP arm.
- This chain gets transferred to the K arm.
- A new malonyl CoA is introduced on the ACP arm.
- The reactions proceed as before. For each cycle the acyl group transferred to the α-carbon of malonyl CoA is 2-carbons longer the previous cycle.
- At the end of 7 cycles a 16 carbon chain is attached to the ACP arm (palmitoyl ACP).
- The C16 unit is hydrolyzed from ACP yielding free palmitate
- Net reaction: Acetyl CoA + 7 malonyl CoA + 14 NADPH + 14 H<sup>+</sup> → Palmitate + 7 CO<sub>2</sub> + 8 CoA + 14 NADP<sup>+</sup> + 6H<sub>2</sub>O



### Differences between FA degradation and synthesis

Characteristic	Degradation	Synthesis
Location	Mitochondrial Matrix	Cytosol
Activated intermediates	Thioesters of CoA	Thioesters of ACP
Enzymes	4 distinct, nonassociated enzymes	FAS is a multienzyme complex
Process	2-Carbon fragments removed as acetyl CoA	2-Carbon elongation using malonyl CoA
Direction	Starts at carboxyl end	Starts at methyl end
Fatty acid size	All sizes are degraded	Only Palmitate is made
Redox reaction cofactors	FAD/FADH <sub>2</sub> and NAD <sup>+</sup> /NADH	NADP <sup>+</sup> /NADPH
Major tissue site	Muscle and liver	Liver
Nutritional status	In starvation	After carbohydrate-rich meal
Hormonal regulation	Low insulin / glucagon ratio	High insulin/glucagon ratio
Activator	FFA generated by hormone-sensitive lipase	Citrate
Inhibitor	Malonyl CoA (inhibits carnitine acyl transferase)	Fatty acyl CoA (inhibits acetyl CoA carboxylase)

### Modification of Palmitic acid

- Palmitic acid is converted to palmitoyl CoA for modification.
- Fatty acids modification takes place by the action of enzyme systems that are present on the cytoplasmic face of the ER membrane.
- FA longer than palmitic acid are synthesized by an elongation enzyme system. Additional carbons are added in 2-carbon units using malonyl CoA as the donor.
- Unsaturated fatty acids are synthesized by the action of specific enzymes called as fatty acid CoA desaturases. The desaturases are specific for specific positions of the double bond.

### Essential Fatty Acids

- Mammals lack the enzymes to introduce double bonds at carbon atoms beyond C9.
- Hence, all fatty acids containing a double bond at positions beyond C9 have to be supplied in the diet. These are called *Essential fatty acids (EFA)*.
- Linoleate (18:2  $\Delta^{9,12}$ ) and Linolenate (18:3  $\Delta^{9,12,15}$ ) are the two essential fatty acids in mammals.
- Other unsaturated fatty acids such as arachidonic acid (20:4  $\Delta^{5,8,11,14}$ ) are derived from these two EFA.
- Eicosanoids are derivatives of arachidonic acid and have hormonal and signaling properties. Classified into prostaglandins, thromboxanes and leukotrienes

### Regulation of FA metabolism

- The two processes of  $\beta$ -oxidation and FA synthesis are coordinately regulated.
- Three hormonal signals determine the state of FA metabolism. Glucagon and epinephrine inhibit FA synthesis and favor oxidation, whereas insulin is anti-lipolytic and stimulates FA biosynthesis.
- The mechanism of hormonal regulation is covalent phosphorylation of acetylCoA carboxylase, the rate-limiting step of FA biosynthesis.
- Acetyl CoA carboxylase is inhibited by phosphorylation. Phosphorylated acetylCoA carboxylase can regain partial activity by allosterically binding citrate.

### Metabolic Enzymes Regulated by Covalent Phosphorylation

<u>Enzyme</u>	<u>Pathway</u>	<u>Reaction Catalyzed</u>	<u>Metabolic Role</u>	<u>Effect of Covalent Phosphorylation</u>
Phosphofructokinase 2	Glycolysis	Formation of F-2,6-BP	catabolic	Inactivation
Pyruvate kinase	Glycolysis	Formation of pyruvate	catabolic	Inactivation
Pyruvate dehydrogenase	Pre-TCA cycle	Formation of acetylCoA	catabolic	Inactivation
Glycogen phosphorylase	Glycogenolysis	Degradation of glycogen	catabolic	Activation
Glycogen synthase	Glycogen synthesis	Formation of glycogen	anabolic	Inactivation
AcetylCoA carboxylase	FA biosynthesis	Formation of malonylCoA	anabolic	Inactivation

## Allosteric Regulation of FA Metabolism

- Acetyl CoA carboxylase is the rate-limiting step of FA biosynthesis. It is allosterically inhibited by palmitoyl CoA and activated by citrate.
- Palmitoyl CoA also inhibits the citrate shuttle and thus slows down FA biosynthesis.
- Malonyl CoA and acetyl CoA inhibit  $\beta$ -oxidation.
- Acetyl CoA inhibits the final step of  $\beta$ -oxidation catalyzed by 3-ketoacyl thiolase.
- Malonyl CoA inhibits the transport of acylCoA to mitochondria via inhibition of carnitine-acyl transferase I.
- Additionally, ATP and NADH also inhibit  $\beta$ -oxidation.

