Regulation of FATTY ACID METABOLISM

Fatty acids—physiological importance

• building blocks ⇒ phospholipids
glycolipids
• covalently attached to proteins ⇒ membrane locations

• derivatives ⇒ hormones (prostaglandins) and intracellular messengers (phosphoinositides)

• important cellular fuels

• stored as triacylglycerols (TAG) in adipose tissue
  ➢ highly concentrated energy stores
  ➢ reduced and anhydrous
### Constituent | $\Delta H$ (kJ·g$^{-1}$ dry wt)
---|---
carbohydrate | 16
fat | 37
protein | 17

**Mobilization of fatty acids**
- Fatty acids are a **major source** of energy for many tissues
  - when glucose availability limited
  - stress
  - prolonged exercise
  - starvation

**In adipose tissues**
- storage as droplets of triacylglycerol
- adipose cells: synthesis
  - storage
  - mobilization

Hormone-sensitive lipase

TAG $\rightarrow$ glycerol + fatty acids
• glycerol $\Rightarrow$ G3P and/or DHAP

• fatty acids form lipoprotein complexes
  ➢ transported via the blood to tissues

• Lipases are hormonally regulated
  o epinephrine
  o norepinephrine
  o glucagon
  o adrenocorticotropic hormone

  ➢ hormones activate adenylate cyclase in adipose cell

\[
\begin{align*}
\uparrow & \text{cAMP} \\
\downarrow & \\
& \text{PKA} \\
& \text{lipase} \quad \text{(active)} \\
& \text{lipase} \quad \text{P}
\end{align*}
\]
Fatty acids are oxidized via $\beta$-oxidation pathway in the mitochondria (FA$\beta$O)

- heart tissue

**Fatty acid biosynthesis (FAS)**
- adipose, liver, intestine, mammary gland
- fatty acid biosynthesis and degradation pathways are distinct
- intermediates of FAS covalently linked to sulfhydryl groups of **acyl carrier protein**
- FAS $\Rightarrow$ cytoplasm
  - FA$\beta$O $\Rightarrow$ mitochondria
- eukaryotes: enzymes of FAS are part of one long polypeptide chain $\Rightarrow$ **fatty acid synthase**
- FAS $\Rightarrow$ NADPH/NADP$^+$
  - FA$\beta$O $\Rightarrow$ NADH/NAD$^+$

*Animals are unable to convert fatty acids into glucose*

- acetyl CoA **cannot** be converted into pyruvate or oxaloacetate
FAS Reactions

Step 1. The **committed step**

- carboxylation of acetyl CoA to form malonyl CoA
- catalyzed by **acetyl CoA carboxylase**

**Acetyl CoA carboxylase**
- contains a biotin prosthetic group covalently linked to the enzyme (via Lys)

**Two stages**
1. Biotin—E + ATP + HCO₃⁻ ⇌ CO₂~biotin—E + ADP + Pᵢ

2. CO₂~biotin—E + acetyl CoA → malonyl CoA + biotin—E

- substrates are bound and products released in a specific sequence
  - ping pong reaction mechanism
Acyl carrier protein (ACP)

- carries the **intermediates** of fatty acid synthesis
- contains a **phosphopantetheine prosthetic group**
  - attached to a Ser of ACP

Elongation cycle in FAS

- enzyme system that catalyzes synthesis of **long fatty acid** chains ⇒ **fatty acid synthase**

**Step 2. Acyl transacylase**
- acetyl group of acetyl CoA is first transferred to ACP

\[
\text{Acetyl CoA + ACP } \leftrightarrow \text{ acetyl-ACP + CoA}
\]

**Step 3. Malonyl transacylase**
- malonyl group of malonyl CoA is transferred to ACP

\[
\text{Malonyl CoA + ACP } \leftrightarrow \text{ malonyl-ACP + CoA}
\]
Step 4. 3-keto acyl transferase (condensing enzyme, CE)

- condensation of acetyl and malonyl units with release of CO₂
- 4 C unit formed from 2C + 3C \( \Rightarrow 4C + CO₂ \) rather than 2C + 2C \( \Rightarrow 4C \)
  - \( \text{driven by } \Delta G° \text{ for decarboxylation} \)
  - although HCO\(_3\)\(^-\) is required for FAS its C does not appear in product

All carbon atoms of fatty acids containing an even number are derived from acetyl CoA

Odd C-fatty acids
- start with propionyl-CoA instead of acetyl CoA

  acetyl transacylase

  propionyl CoA \( \Leftrightarrow \) propionyl-ACP + CoA

- then link with malonyl using CE
Step 5. 3-ketoacyl-ACP reductase
- acetoacyl-ACP is reduced to D-3-hydroxybutyryl-ACP using NADPH

Step 6. 3-hydroxyacyl-ACP dehydratase
- D-3-butyryl-ACP is dehydrated to form crotonyl-ACP (trans-Δ²-enoynl-ACP)

Step 7. Enoyl-ACP reductase
- crotonyl-ACP is reduced to butyryl-ACP
- completes one turn of fatty acid synthesis

⇒ C4 acyl-ACP

- Cycle continues
  - 2C unit added each turn (from malonyl)
  - continues until chain is 16-C long
  - 3-ketoacyl-ACP synthase cannot accommodate larger substrates than 16-C
Final step—Palmitoyl thioesterase
\[ C_{16}\text{-acyl-ACP} + H_2O \rightarrow \text{palmitate} + \text{ACP-SH} \]

Stoichiometry of FAS

Synthesis of palmitate
\[ \text{Acetyl CoA} + 7 \text{ malonyl CoA} + 14 \text{ NADPH} + 14H^+ \rightarrow \text{palmitate} + 7\text{CO}_2 + 14 \text{NADP}^+ + 8\text{CoA} + 6\text{H}_2\text{O} \]

Synthesis of malonyl-CoA used for palmitate
\[ 7 \text{acetyl CoA} + 7\text{CO}_2 + 7\text{ATP} \rightarrow 7 \text{malonyl CoA} + 7\text{ADP} + 7\text{Pi} + 7H^+ \]

Overall
\[ 8 \text{acetyl CoA} + 7 \text{ATP} + 14\text{NADPH} + 7H^+ \rightarrow \text{palmitate} + 14 \text{NADP}^+ + 8\text{CoA} + 6\text{H}_2\text{O} + 7\text{ADP} + 7\text{Pi} \]

In eukaryotes ⇒ elongation and unsaturation of fatty acids occurs on cytoplasmic face of ER
• Mammalian fatty acid synthase

• A multienzyme complex
  ➢ homodimer of 260 KDa subunits

  ➢ each chain ⇒ 3 domains joined by very flexible regions

  ➢ enzymes catalyzing steps 2-7 and a thioesterase

  ➢ 7 different catalytic activities on a single polypeptide chain

Advantages of complex
  o synthesis of different enzymes is coordinated
  o enzyme complex is more stable than separate enzymes
  o intermediates can be efficiently “handed” from one active site to another
  o side reactions are reduced
• Domain 1
  - substrate entry and condensation unit
    - acetyl transacylase
    - malonyl transacylase
    - 3-ketoacyl synthase (CE)
  - the binding of acetyl and malonyl building blocks and condensation of these units

• Domain 2
  - the reduction unit
    - Acyl carrier protein
    - 3-ketoacyl reductase
    - dehydratase
    - enoyl reductase

• Domain 3
  - palmitate release unit
    - palmitoyl thioesterase
  - liberation of palmitate when the growing acyl chain reaches its limit length of 16-C
Flexible Phosphopantetheine Unit

• FAS begins ⇒ attachment of acetyl of acetyl CoA to Ser in **acetyl transacylase**

• malonyl of malonyl CoA also becomes O-linked to **malonyl transacylase**

• occur in **domain 1 of synthase**

• acetyl unit is transferred to the Cys of **condensing enzyme (CE)**

• malonyl unit is transferred to the phosphopantetheine unit of ACP of the **other chain** in the dimer

• **domain 1** of each chain interacts with **domains 2 and 3** of the other chain

• elongation involves the joining of the acetyl unit on **CE** to a 2-C portion of the malonyl unit on ACP
• CO$_2$ is released and an **acetoacetyl-S-phosphopantetheine** unit is formed on **ACP**
  
  o active site S-H on **CE** is restored

• acetoacetyl group is delivered to 3 active sites in domain 2 of the opposite chain to reduce it to a butyryl unit

• C4 unit migrates from **phosphopantetheine** sulfur on **ACP** to the Cys sulfur on the **CE**
  
  o synthase is ready for another cycle

• butyryl unit on **CE** becomes linked to a 2-C part of the malonyl unit on **ACP** to form a 6-C unit on **ACP**
  
  o 6-C unit undergoes reduction

• 5 additional rounds of condensation and reduction produce a palmitoyl (C16) chain on **CE**
• palmitate is produced by hydrolysis reaction by thioesterase on **domain 3**

• **flexibility and 20 Å maximal length of the phosphopantetheine unit** are **critical** for function of multienzyme complex

  - enzyme subunits don’t undergo large structural rearrangements
  - substrate is a **long flexible arm** that can reach numerous active sites

**Role of Citrate**

• synthesis of palmitate requires
  - 8 molecules of acetyl CoA
  - 14 NADPH
  - 7 ATP

• FAS occurs in cytoplasm whereas **acetyl CoA** is formed from pyruvate in mitochondria
• acetyl CoA transferred from mitochondria to cytoplasm **but is not permeable** to acetyl CoA

  o barrier to acetyl CoA is bypassed by citrate which carries acetyl groups across the inner mitochondrial membrane

  o citrate formed by condensation of acetyl CoA with oxaloacetate in mitochondrial matrix

  o citrate transported to cytoplasm is cleaved by **ATP citrate lyase**

\[
\text{Citrate} + \text{ATP} + \text{CoA} + \text{H}_2\text{O} \rightarrow \text{acetyl CoA} + \text{ADP} + \text{P}_i + \text{oxaloacetate}
\]

**Acetyl CoA Carboxylase (ACC)**

• fatty acid metabolism is stringently controlled so that synthesis and degradation are responsive to metabolic needs
• FAS is **maximal** when CHO and energy are plentiful and fatty acids are scarce

• ACC plays a **key role** in regulation of fatty acid metabolism
  
  o catalyzes the **committed step** in FAS

• ACC controlled by 3 global signals
  o glucagon
  o epinephrine
  o insulin

  o control also exerted by levels of
    ▪ citrate
    ▪ palmitoyl CoA
    ▪ AMP

• ACC subject to regulation by covalent modification
  o Ser is phosphorylated by an **AMP-activated protein kinase (APK)**
- APK is stimulated by AMP and inhibited by ATP
  - dephosphorylated by **protein phosphatase-2A (PP-2A)**

epinephrine and glucagon

\[ \text{PKA} \]

\[ \text{PP-2A} \]  \( \rightarrow \)  \[ \text{PP-2A} \]  \( \text{P} \)

- (active)

\[ \text{pp-1} \]  \( \rightarrow \)  \[ \text{P_i}, \text{H}_2\text{O} \]

- (inactive)

insulin

**Citrate**
- partially reverses inhibition by phosphorylation
- high citrate \( \Rightarrow \) 2-C units and ATP are available for FAS
**Palmitoyl CoA**
- antagonizes effect of citrate
- indicates an **excess** of fatty acids
- inhibits citrate transport from mitochondria by translocase
- inhibits glucose-6-P dehydrogenase

**Regulation of FAS and FAβO**

- FAS and FAβO are reciprocally regulated
  - **starvation** ⇒ epinephrine and glucagon stimulate **adipose cell lipase** (lipolysis)
    - provides free fatty acids for β-oxidation
  - **fed state** ⇒ insulin inhibits **adipose cell lipase** (lipolysis)
  - malonyl CoA inhibits carnitine acyltransferase
    - fatty acyl CoAs do not have ready access to mitochondria (β-oxidation) in times of plenty