Slide 2

Fatty Acids as Energy Source

- Triglycerides yield 37 kJ/g dry weight
- Protein 17 kJ/g
- Glycogen 16 kJ/g (even less wet weight)
- Total stored energy in body (Table 24.1)
  - Fat ~555,000 kJ
  - Protein ~ 102,000 kJ
  - Glycogen ~ 3,000 kJ
- More reduced than carbohydrate

Slide 3

Fatty Acids as Energy Source, con’t.

- Major Sources of Fatty Acids
  - Stored Fat (Adipose Tissue)
  - Dietary Fat
  - Biosynthetic Fat (from glucose in liver)
- Low solubility of Triglyceride and Fatty Acids require special transport mechanisms involving lipoproteins
Slide 4

Adipose Tissue Triglycerides

- Triglycerides hydrolyzed by hormone sensitive lipase
  - Hormonal (epinephrine, glucagon, ACTH) stimulation activates the cyclic AMP pathway
  - Fatty acids and glycerol released to the blood
  - Fatty acids bound to serum albumin for transport in blood
- See Fig 24.2

Slide 5

Dietary Triglycerides

- Mixed with bile salts to form micelles
- Hydrolyzed in the duodenum by pancreatic lipase to fatty acids plus monoglycerides
- Micelles adsorbed into epithelial cells where triglycerides are resynthesized and packaged into chylomicrons, which are released into the lymphatic system, then the blood
  - Short chain fatty acids are transported directly to the portal vein.
  (See Fig 24.3 and 24.4)

Slide 6

Biosynthetic Triglycerides

- Made in the liver from carbohydrate
- Exported as part of a lipoprotein called very low density lipoprotein (VLDL)
  - (VLDL is discussed in Section 25.5, page 840)
- Triglycerides from both VLDL and chylomicrons are hydrolyzed in the blood by lipoprotein lipase, releasing free fatty acids (FFA) to tissues

Binding to serum albumin helps to minimize the detergent properties of fatty acids, which otherwise might be strong enough to disrupt cellular membranes.

Lipoprotein lipase is attached to the surface of blood vessels in tissues. The attachment can be released by administration of heparin.
Fatty Acid Activation

- Once fatty acids get into the cell, they are immediately activated to thiol esters of coenzyme A.
  - This costs the equivalent of 2 ATP (Fig 24.7)
- Oxidation occurs in the mitochondria, but CoASH esters cannot cross the mitochondrial membrane.

Role of Carnitine in Fatty Acid Oxidation

- To cross the mitochondrial membrane, fatty acids are transesterified to form esters of the amino acid carnitine.
  - The enzyme is carnitine acyltransferase.
  - A carnitine/acylcarnitine antiport transport protein transports the acyl carnitine across the inner mitochondrial membrane.
  - Carnitine acyl transferase in the mitochondria re-forms the fatty acyl-CoA (See Fig 24.9).

Beta Oxidation

- Franz Knoop’s early labeling experiments established that fatty acids are degraded two carbons at a time.
  - Cleavage occurs at the beta-carbon, hence the term beta oxidation.
  - A series of phenyl derivatives of fatty acids with different chain lengths produced either phenyl acetate or benzoate as excreted products. (See Fig 24.5)
Activation of Short Chain Fatty Acids

- Short chain acids can bypass the cytoplasmic activation and enter the mitochondria directly.
- They are activated by a transfer of CoASH from succinyl CoA

\[
\text{R}^0\text{CH}_2\text{CH}_2\text{CO}\text{SH} + \text{HO}\text{CCH}_2\text{CH}_2\text{SCoA} \rightarrow \text{R}^0\text{CCH}_2\text{CH}_2\text{OSOH} + \text{HO}\text{CCH}_2\text{CH}_2\text{CoA}
\]

Beta Oxidation Spiral

- A series of four reactions that results in shortening the chain by two carbons
- First three reactions analogous to reactions of the TCA cycle:

\[
\text{HO}\text{CCH}_2\text{CH}_2\text{OSCoA} \rightarrow \text{HO}\text{CCH}_2\text{CH}_2\text{OSCoA} \rightarrow \text{HO}\text{CCH}_2\text{CH}_2\text{OSCoA} \rightarrow \text{HO}\text{CCH}_2\text{CH}_2\text{OSCoA}
\]

Acyl-CoA Dehydrogenase

- A family of three soluble matrix enzymes
- All are flavoproteins with differing chain length specificity (long, medium, short)
- Electrons passed to an electron transfer flavoprotein (ETF), then via an Fe/S protein to Coenzyme Q
  - See Fig 24.11
  - Enzyme inhibited by Hypoglycin from akee fruit. (Fig 24.14)

Note this pair of electrons would yield 1.5 ATP’s when reduced coenzyme Q is reoxidized by the electron transport chain.
Enoyl-CoA Hydratase

- Also called crotonase
- Converts trans enoyl CoA ester to the L-beta hydroxy acyl-CoA ester
- Enzymes with other specificity also found
  - (See Fig 24.15)

Hydroxyacyl-CoA Dehydrogenase

- Oxidizes L-hydroxy to keto
- NAD is the electron acceptor
  - Reoxidation of the NADH can produce 2.5 ATP
- See Fig 24.16

Thiolase
(or beta-ketothiolase)

- Thiolytic cleavage of C-C bond
  - Cysteine SH on enzyme first attacks the carbonyl, cleaving the alpha-beta bond
  - Acyl group then transferred to CoASH
  - See Fig 24.17
- Overall reaction is a “reverse Claisen condensation”
- Reaction is reversible
- Products are acetyl-CoA and fatty acyl-CoA two carbons shorter
Beta Oxidation Summary

- Each turn of the “spiral” produces an acetyl-CoA, CoQH$_2$, and NADH
- Palmitic Acid (C$_{16}$)
  - 8 Acetyl-CoA, 7 CoQH$_2$, 7 NADH
- Stearic Acid (C$_{18}$)
  - 9 Acetyl-CoA, 8 CoQH$_2$, 8 NADH
- Acetyl-CoA can be oxidized by TCA cycle

Odd Chain Fatty Acids

- Last unit is propionyl CoA
- Three reactions convert propionyl-CoA to succinyl-CoA (Fig 24.19)
  - Propionyl-CoA carboxylase
    - A biotin enzyme
  - Methylmalonyl-CoA epimerase
  - Methylmalonyl-CoA mutase
    - A B$_{12}$ enzyme (See Fig 24.21 and Page 793)

Unsaturated Fatty Acids

- As chain is degraded, double bond ends up in wrong place and must be isomerized.
- Extra double bonds in polyunsaturated fatty acids also require special enzymes.
- See Fig 24.23 and 24.24
- Don’t worry about details

Compare the biotin mechanism with pyruvate carboxylase (an anaplerotic reaction and a gluconeogenic enzyme) and acetyl-CoA carboxylase, which we will discuss in the next chapter.
Peroxisomal Oxidation

- Takes place in peroxisomes
- Initial double bond formation is by an acyl-CoA oxidase containing FAD
- FADH$_2$ of the oxidase is reoxidized by oxygen, producing hydrogen peroxide
  - Fig 24.25

Branch Chain Fatty Acids

- Phytanic acid has CH$_3$ group on beta-carbon, so one could not produce a keto group there.
- Oxidation at the alpha carbon by a hydroxylase can cleave one carbon
- The process is called alpha oxidation
- Also occurs in brain fatty acids producing some alpha hydroxy and odd chain fatty acids.
- Defect in pathway found in Refsum’s Disease in which phytanic acid accumulates.
  - Fig 24.26

Synthesis of Acetoacetate

- “Burning” acetyl-CoA requires oxaloacetate. (OAA)
- When OAA concentrations are low, acetyl-CoA can build up.
- Fatty acid oxidation would stop when all of the cell’s CoASH is tied up as acetyl-CoA.
- How can the cell release the CoA?
Synthesis of Acetoacetate, con’t.

- **Thiolase** is reversible.
- As acetyl-CoA builds up, so does acetoacetyl-CoA.
- Cleavage of acetoacetyl-CoA can liberate CoASH, producing acetoacetate.
- While simple hydrolysis would accomplish that, it doesn’t work that way.

Note the addition of the methyl group of acetyl-CoA to a carbonyl carbon, coupled to the hydrolysis of the thiol ester bond.
Synthesis of Acetoacetate, con’t.

- The sum of these two reactions is the same as the hydrolysis of acetoacetyl-CoA.

\[
\text{acetoacetyl-CoA} + \text{acetyl-CoA} \rightarrow \text{HMG-CoA} + \text{CoASH} \\
\text{HMG-CoA} \rightarrow \text{acetoacetate} + \text{acetyl-CoA} \\
\text{sum:} \\
\text{acetoacetyl-CoA} \rightarrow \text{acetoacetate} + \text{CoASH}
\]

Metabolism of Acetoacetate

- The liver excretes acetoacetate from fatty acid breakdown as a fuel for other tissues.
- Acetoacetate is taken up in other tissues, enters the mitochondria, and is activated by 3-ketoacyl-CoA transferase.

\[
\begin{align*}
&\text{H}_3\text{C} &\text{CH}_2 &\text{CH}_2 &\text{OH} \\
&\text{HO} &\text{CH}_2 &\text{CH}_2 &\text{SCoA} \\
&+ &\text{3-Ketoacyl-CoA} \\
&\text{Transferase} &\text{H}_3\text{C} &\text{CH}_2 &\text{CH}_2 &\text{SCoA} \\
&\text{HO} &\text{CH}_2 &\text{CH}_2 &\text{OH}
\end{align*}
\]

Note this reaction bypasses the synthesis of a GTP in the mitochondria, so the cost of activation of the acetoacetate is equivalent to one GTP (or one ATP).

Metabolism of Acetoacetate, con’t.

- Acetoacetyl-CoA is broken down by thiolase to acetyl-CoA, and the acetyl-CoA burned in the TCA cycle in peripheral tissues.
- Liver lacks the enzyme 3-ketoacyl-CoA transferase, so it cannot re-activate acetoacetate once it is formed.
Ketone Body Formation

• When oxaloacetate is low, the acetyl-CoA cannot be metabolized, so acetoacetate builds up.
• Alternative reactions of acetoacetate include reduction and decarboxylation.

\[
\begin{align*}
\text{NAD} & \quad \overset{\text{reduction}}{\rightarrow} \quad \text{H}_2\text{C} = \text{CH}\text{COOH} \\
\text{OH} & \quad \overset{\text{decarboxylation}}{\rightarrow} \quad \text{H}_2\text{O} = \text{C} = \text{CH}_3 + \text{CO}_2
\end{align*}
\]

The three compounds acetoacetate, beta-hydroxybutyrate, and acetone constitute what are called ketone bodies.

Ketone Body Formation

• Accumulation occurs when fatty acids are broken down for energy in absence of sufficient carbohydrate to make OAA.
• Occurs in starvation.
• Occurs in high fat diets (eating eskimo diet without adaptation to it).
• Occurs in diabetes, where cells are “starved” for glucose because of lack of insulin.
• Ketosis can lead to drop in blood pH.

The state of ketosis can often be detected by the odor of acetone on the breath. The lowered pH can lead to acidosis, which can be a dangerous condition.