Midterm 2 (in class), TUE (5/19)

Bring Calculator to Exam!

Room Assignments (Last Name Initial)

A – N  198 Young Hall
O – Z  180 Medical Sciences C
Review Sessions

• Friday (5/15): Kleiber Hall, 5-7 p.m.
• Monday (5/18): Kleiber Hall, 5-7 p.m.
• ESSENTIALS posted
• Problem Sets (3) 4-6

Abel Office Hours: Tomorrow (FRI)
(8:30 – 10 am)
(4 – 5 pm)
Lecture 14

Lipid Metabolism

• \(\beta\)-Oxidation of fatty acids
• Ketogenesis
• Lipid biosynthesis
**Activation and Transport of Fatty Acids**

**Fatty Acid Activation (Cytosol)**

\[
\text{Fatty Acid} + \text{CoA} + \text{ATP} \rightleftharpoons \text{Acyl-CoA} + \text{AMP} + \text{PPi} + \text{H}_2\text{O} \rightarrow 2 \text{Pi}
\]

**Mitochondrial Matrix**

- **Carnitine acyltransferase I**
- **Carnitine acyltransferase II**
- **β-Oxidation**

Diagram:

- **Outer Membrane**
  - Acyl-CoA
  - CoA-SH
  - Carnitine
  - O-Acyl Carnitine

- **Intermembrane Space**
  - Carnitine

- **Inner Membrane**
  - Transporter
  - Acyl-CoA
  - CoA-SH
I. Fatty Acid Activation (Acyl-CoA)

II. Successive $\beta$-Oxidation of Acyl-CoA

1. Oxidation ($\rightarrow$ FADH$_2$)
2. Hydration (+ H$_2$O)
3. Oxidation ($\rightarrow$ NADH)
4. Cleavage (+ CoA, $\rightarrow$ Acetyl-CoA)
**β-Oxidation of Saturated Fatty Acids**

1. **Acyl-CoA:**
   - **Acyl-CoA:**
   - **Acyl-CoA FAD oxidoreductase**
   - **Acyl-CoA Dehydrogenase**

2. **Trans-Δ²-Enoyl CoA:**
   - **L-3-Hydroxyacyl CoA hydratase**
   - **Enoyl-CoA Hydratase**

3. **L-3(β)-Hydroxyacyl CoA:**
   - **L-3-Hydroxyacyl CoA:NAD⁺ oxidoreductase**
   - **β-Hydroxyacyl-CoA Dehydrogenase**

4. **3(β)-Ketoacyl CoA:**
   - **3-Keto acyl CoA: CoA acyltransferase**
   - **Acyl-CoA acetyltransferase**
   - **Acyl CoA (Thiolase)**

**Mitochondrial matrix**

Additional rounds of β-oxidation

Review
**ATP Yield**

148 ATP – 2 ATP (fatty acid activation) = **146 ATP per 18:0**

8.2 ATP per carbon fatty acid

Compare to glucose oxidation: 38 ATP per hexose

6.3 ATP per carbon carbohydrate

**Water Yield**

52 H₂O – 18 H₂O (TCA) – 8 H₂O (β-Oxidation) = **26 H₂O per 18:0**

~1.6 g H₂O per gram fatty acid
**β-Oxidation of Unsaturated Fatty Acids**

3 turns of β-oxidation

3 Acetyl CoA

Enoyl CoA isomerase

β-oxidation step 2 (skip step 1)
**β-Oxidation of Unsaturated Fatty Acids**

- β-oxidation step 3
  - NAD$^+$ to NADH + H$^+$
  - Formation of 1 Acetyl CoA

- β-oxidation step 4
  - Conversion of CoA
  - FAD to FADH$_2$

- β-oxidation step 1
  - trans-$\Delta^2$, cis-$\Delta^4$

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\[ \beta\text{-Oxidation of Unsaturated Fatty Acids} \]

\[ \beta\text{-oxidation step 1} \]

\[ 18^\text{H}3\text{C} \]

\[ \text{2,4-Dienoyl-CoA reductase} \]

\[ \text{NADPH + H}^+ \]

\[ \text{NADP}^+ \]

\[ \text{Enoyl CoA isomerase} \]

\[ \beta\text{-oxidation starting with step 2,} \]
\[ \text{then 4 complete cycles} \]

\[ 5 \text{ Acetyl CoA} \]

\[ \text{trans-}\Delta^2, \text{ cis}\Delta^4 \]

\[ \text{trans-}\Delta^3 \]

\[ \text{trans-}\Delta^2 \]
**β-Oxidation of Unsaturated Fatty Acids: Summary**

Linoleic Acid: \(18:2 (\Delta^9,12)\) after 3 regular rounds of β-oxidation

Enoyl CoA isomerase

Enoyl CoA isomerase  \(\text{cis-}\Delta^3\) (or \(\text{trans-}\Delta^3\)) → \(\text{trans-}\Delta^2\)

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Fate of Propionyl-CoA

Propionyl CoA

D-Methyl malonyl CoA

L-Methyl malonyl CoA

Succinyl CoA

Glucose

PEP + CO₂

PEP-CK

TCA Cycle

OAA

“4CO₂”

“Left-over” from the β-oxidation of odd-numbered fatty acids

1.

H₃C—C—C—O

SCoA

+ HCO₃⁻ + ATP

2.

H₃C—H—C—C—O

SCoA

+ HPO₄²⁻ + ADP

3.

H₂C—H—C—C—O

SCoA

Hope this helps!
Cobalamin (vitamin B12)

5,6-di-methylbenzimidazole ribonucleotide
Robert B. Woodward (Nobel Prize, 1965)
Ketogenesis

Synthesis of “Ketone Bodies” from Acetyl-CoA under certain physiological and pathological conditions:

- Starvation
- Diabetes mellitus
Glycogen (muscles, liver) is already depleted!

Starvation

Protein → Pyruvate → Acetyl-CoA

Gluconeogenesis

Glucose → Oxaloacetate → TCA Cycle

TCA Cycle: Citrate, Isocitrate, α-Ketoglutarate, Succinyl-CoA, Malate, Fumarate
Pyruvate

Succinyl-CoA

$\alpha$-Ketoglutarate

Fumarate

Isocitrate

Malate

Glucose

Oxaloacetate

Citrate

FADH$_2$

NADH

ETC

Fatty Acids

TCA Cycle

Acetyl-CoA

α-Ketoglutarate

Succinyl-CoA

(Severe) Starvation

Protein

Ketone Bodies

CoA

Ketone Bodies

CoA

Fatty Acids

ATP

ETC

CoA

Google Man II
Diabetes mellitus

- “Excessive excretion of sweet urine” (glucosuria)
- Metabolic disease caused by a deficiency in the secretion or action of insulin (6-10 million in the US)
  - Type I: insufficient insulin production (10% of cases)
  - Type II: insulin-insensitivity (defective response, 90%)
- Many complications
  - Glycation (non-enzymatic protein modification)
  - Cataracts (glucose $\rightarrow$ sorbitol, rupture of lens cells)
  - Excessive and incomplete $\beta$-oxidation (ketogenesis)
Insufficient glucose uptake: cells are “starved”

Diabetes mellitus

TCA Cycle

Glucose

Pyruvate

Acetyl-CoA

Fumarate

Isocitrate

Malate

CoA

Oxalaoacetate

Citrate

Fatty Acids

NADH

FADH₂

ETC

ATP

Protein

Ketone Bodies

Diabetes mellitus

Ketone Bodies

Insufficient glucose uptake: cells are “starved”
Fate of Excess Acetyl-CoA

2 x \( \text{Acetyl CoA} \)

\( \beta \)-Ketoacyl thiolase

\( \text{Acetoacetyl CoA} \)

\( \text{Acetyl CoA} \) and CoASH

\( \beta \)-hydroxy-\( \beta \)-methyl glutaryl CoA (HMG-CoA)
**Brain (Starvation)**

**Muscles**

**Blood**

**Danger of Acidosis (Ketosis)**

**Blood**

**NADH + H^+**

**β-Hydroxybutyrate dehydrogenase**

**Acetoacetate**

**Acetone**

**β-hydroxy-β-methyl glutaryl CoA (HMG-CoA)**

**HMG-CoA lyase**

**Ketone Bodies**

**p. 79**
Cut-off for Second Midterm
(show video clip)
Lipid Biosynthesis
(Cytosol)
Biosynthetic Functions of the TCA Cycle

PEP → Pyruvate → Acetyl-CoA → Succinyl-CoA → α-Ketoglutarate → Fumarate → Isocitrate → Malate → Oxaloacetate → Citrate → α-Ketoglutarate (catalyzed by succinyl-CoA) → Fumarate → Malate → Oxaloacetate → Citrate

Pyrimidine Bases

Thr → Asp → Ile → Met → Asn → Lys

Fatty Acids

Steroids

Acetyl-CoA (cytosol)

Glutathione

Ornithine → Lys → Arg

Glutamine (Gln) → Pro → Gln

Purine Bases

Porphyrines

Heme → Chlorophyll → Vitamin B₁₂
The Citrate Shuttle

MITOCHONDRIA

CYTOSOL

Acetyl CoA

CITRATE

MALATE

PYRUVATE

Fatty acid synthesis

NADH

NADPH + H+ + CO2

NADPH + H+ + CO2

MALATE

PYRUVATE

NADP+  NAD+  NADH

OAA

1  2  3  4  5  6  7

Pentose-P Pathway

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Biosynthesis of Saturated Fatty Acids

Activation of Acetyl-CoA

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{carboxyl} \\
\text{C} & \quad \text{group} \\
\text{S} & \quad \text{thioester} \\
\text{CoA} & \\
\end{align*}
\]

+ \quad \text{HCO}_3^- \\
\quad + \quad \text{ATP} \\
\text{Acetyl-CoA Carboxylase} \\
\text{H}_3\text{C} & \quad \text{carboxyl} \\
\text{C} & \quad \text{group} \\
\text{S} & \quad \text{thioester} \\
\text{CoA} & \\
\text{H} & \quad \text{acyl} \\
\text{group} \\
\text{CoA} & \\
\text{O} & \quad \text{phosphate} \\
\text{HPO}_4^{2-} & \\
\end{align*}
\]

Transfer of Malonyl-CoA onto an “Acyl Carrier Protein (ACP)”

\[
\begin{align*}
\text{O} & \quad \text{carboxyl} \\
\text{C} & \quad \text{group} \\
\text{O} & \quad \text{thiol ester} \\
\text{CoA} & \\
\text{H} & \quad \text{acyl} \\
\text{group} \\
\text{CoA} & \\
\text{O} & \quad \text{phosphate} \\
\text{HPO}_4^{2-} & \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{carboxyl} \\
\text{C} & \quad \text{group} \\
\text{O} & \quad \text{thiol ester} \\
\text{CoA} & \\
\text{H} & \quad \text{acyl} \\
\text{group} \\
\text{ACP} & \\
\text{O} & \quad \text{phosphate} \\
\text{HPO}_4^{2-} & \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{carboxyl} \\
\text{C} & \quad \text{group} \\
\text{O} & \quad \text{thiol ester} \\
\text{ACP} & \\
\text{H} & \quad \text{acyl} \\
\text{group} \\
\text{CoASH} & \\
\text{O} & \quad \text{phosphate} \\
\text{HPO}_4^{2-} & \\
\end{align*}
\]
Transfer of Acetyl-CoA onto a second Acyl Carrier Protein (ACP)

\[
\text{Acetyl-CoA:ACP transacylase} \\
H_3C-\text{C}S\text{-CoA} + \text{ACP} \xrightarrow{\text{enzyme}} H_3C-\text{C}S\text{-ACP} + \text{CoASH}
\]

- **Acetyl CoA**
- **Acyl carrier protein**
- **Acetyl ACP**

**Diagram:**
- Acetyl-ACP
- Malonyl-ACP

**Enzymes:**
- KS
- AT
- ER
- HD
- MT
- KR
Coenzyme A (CoA-SH)

Pantothenic Acid (Vitamin B₅)

Malonyl groups are esterified to the —SH group.
Simple Model of the **Fatty Acid Synthase Complex**
β-Ketobutyryl-ACP

Diagram showing the β-Ketobutyryl-ACP enzyme with a chemical structure and the addition of CO₂.
Use of growing chain instead of acetyl ACP in next rounds.

1. \( \beta \)-Ketoacyl ACP synthase
   - Acetoacetyl ACP (Acetoacyl ACP)
2. \( \beta \)-Ketoacyl ACP reductase
   - D-3(\( \beta \))-hydroxyacyl ACP
3. 3-Hydroxyacyl ACP dehydratase
   - 3 (\( \beta \))-ketoacyl ACP
4. Enoyl ACP reductase
   - Butyryl ACP
Use of growing chain instead of acetyl ACP in next rounds

1. \(\beta\)-Ketoacyl ACP synthase
   \[ \text{Acetoacetyl ACP} \to \beta\text{-Ketoacyl ACP} \]

2. \(\beta\)-Ketoacyl ACP reductase
   \[ \text{NADPH} + H^+ + \beta\text{-Ketoacyl ACP} \to \text{NADP}^+ + \text{D-3(\(\beta\))-hydroxyacyl ACP} \]

3. 3-Hydroxyacyl ACP dehydratase
   \[ \text{H}_2\text{O} + \text{D-3(\(\beta\))-hydroxyacyl ACP} \to \text{3 (\(\beta\)-ketoacyl ACP} \]

4. Enoyl ACP reductase
   \[ \text{NADPH} + H^+ + \text{3 (\(\beta\)-ketoacyl ACP} \to \text{NADP}^+ + \text{Butyryl ACP} \]
I.  Activation of Acetyl-CoA (Malonyl-ACP)

II.  Successive Condensation of Acetyl-Units

1. Condensation (\(\rightarrow \text{CO}_2\))
2. Reduction (+ NADPH)
3. Dehydration (\(\rightarrow \text{H}_2\text{O}\))
4. Reduction (+ NADPH)

Acyl\((\text{C+2})\)-ACP

Typically stops at 16:0 (Palmitoyl-CoA)
Activation

\[
7\text{Acetyl-CoA} + 7\text{CO}_2 + 7\text{ATP} \rightarrow 7\text{Malonyl-CoA} + 7\text{ADP} + 7\text{Pi}
\]

Biosynthesis (7 rounds)

\[
\text{Acetyl-CoA} + 7\text{Malonyl-CoA} + 14\text{NADPH} \rightarrow \text{Palmitoyl-CoA (16:0)} + 7\text{CO}_2 + 7\text{CoA} + 14\text{NADP}^+ + 7\text{H}_2\text{O}
\]

Sum

\[
8\text{Acetyl-CoA} + 7\text{ATP} + 14\text{NADPH} \rightarrow \\
16:0 \text{ (free acid)} + 8\text{CoA} + 7\text{ADP} + 7\text{Pi} + 14 \text{ NADP}^+ + 6\text{H}_2\text{O}
\]