1) Lipid Digestion, Absorption and Transport

Major form of energy: triacylglycerol/fat/triglycerides

- 90% of dietary lipid
- oxidized to $CO_2$ and $H_2O$
- 6 times more energy/weight of glycogen
- water insoluble
- emulsified by bile salts/bile acids in small intestine
- digestion at lipid/water interface
- cut at pos 1 and 3 by lipase (triacylglycerol lipase)
  $TAG \rightarrow 1,2$-diacylglycerol $\rightarrow 2$-acylglycerol
- FA uptake by enterocytes, bind to I-FABP
Energy Content of Food Constituents

<table>
<thead>
<tr>
<th>Constituent</th>
<th>$\Delta H$(kJ · g$^{-1}$ dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>16</td>
</tr>
<tr>
<td>Fat</td>
<td>37</td>
</tr>
<tr>
<td>Protein</td>
<td>17</td>
</tr>
</tbody>
</table>


Fat storage: anhydrous! $\Rightarrow$ Up to 10x more energy per weight than hydrated glycogen

$\begin{align*}
\text{1-Palmitoyl-2,3-dioleoyl-glycerol}
\end{align*}$
Bile acids

- have detergent character to help solubilize and absorb lipids in the gut
- made in the liver, secreted as glycin or taurine conjugates into the gallbladder for storage
- from gallbladder secreted into small intestine, where lipid digestion and absorption mainly takes place
Mechanism of interfacial activation of triacylglycerol lipase in complex with procolipase

- **Pancreas Lipase** = TAG lipase
- Degrades TAG to 2-acylglycerol
- Lipase activation by colipase
- Interfacial activation
- Activity depends on surface area
- Alpha/beta hydrolase fold
- 25 AS lid structure
- Catalytic triad, Asp-Ser-His, related to serine proteases
- Hydrolysis similar to peptidase
Orlistat

- Xenical, Roche (tetrahydrolipstatin)

- treat obesity by inhibiting lipid absorption -> reduce caloric intake

- Inhibits pancreas lipase

- side effect, oily and loose stool

- Recommended “to bring a change of clothes with you to work”

- Avoid high fat food!
Substrate binding to phospholipase $A_2$

- No interfacial activation
- No conformational change
- Upon interfacial binding
- Why ??
LIPID ABSORPTION by enterocytes

As micelles with bile salts and PC (lecithin)
Or lipid-protein complexes also for Vit A, D, E, K

Inside the cell:
• **I-FABP**, increases solubility of FAs in the cytosol of **enterocytes**
• Protect cells from their detergent effect
• β-clam structure (Muschel)

X-Ray structure of rat intestinal fatty acid-binding protein
B) Lipids are transported as Lipoproteins

• How does an organism transport water insoluble substances, i.e. lipids?

• In form of lipid/protein complexes, lipoproteins

• The protein wraps around a lipid droplet and thereby makes it soluble
The fate of dietary lipids

- Hydrolyzed lipids are absorbed by the intestinal mucosa
- Converted back to triglycerides!
- Packed into lipoprotein particles, chylomicrons
- Released into lymph/blood -> delivered to tissue
- Triglyceride made by liver is packaged into VLDL part. ->
- Released into blood
- TAG hydrolyzed in periphery by lipoprotein lipase ->
- FA uptake but glycerol back transport to liver and kidney
- TAG in adipose tissue is mobilized by hormone-sensitive lipase -> free FA enter blood, bound to serum albumin
Different types of lipoproteins

- **Chylomicrons**, transport from intestine through lymphatic vessels into blood/periphery
- **VLDL, IDL, and LDL** made by the liver to transport endogenous lipids to periphery
- HDL transport cholesterol from the periphery back to liver
- The more lipids the **lower the density** of the lipoprotein particle
Different types of lipoproteins (2)

<table>
<thead>
<tr>
<th></th>
<th>Chylomicrons</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (g \cdot cm(^{-3}))</td>
<td>&lt;0.95</td>
<td>&lt;1.006</td>
<td>1.006–1.019</td>
<td>1.019–1.063</td>
<td>1.063–1.210</td>
</tr>
<tr>
<td>Particle diameter (Å)</td>
<td>750–12,000</td>
<td>300–800</td>
<td>250–350</td>
<td>180–250</td>
<td>50–120</td>
</tr>
<tr>
<td>Particle mass (kD)</td>
<td>400,000</td>
<td>10,000–80,000</td>
<td>5000–10,000</td>
<td>2300</td>
<td>175–360</td>
</tr>
<tr>
<td>% Protein(^a)</td>
<td>1.5–2.5</td>
<td>5–10</td>
<td>15–20</td>
<td>20–25</td>
<td>40–55</td>
</tr>
<tr>
<td>% Phospholipids(^a)</td>
<td>7–9</td>
<td>15–20</td>
<td>22</td>
<td>15–20</td>
<td>20–35</td>
</tr>
<tr>
<td>% Free cholesterol(^a)</td>
<td>1–3</td>
<td>5–10</td>
<td>8</td>
<td>7–10</td>
<td>3–4</td>
</tr>
<tr>
<td>% Triacylglycerols(^b)</td>
<td>84–89</td>
<td>50–65</td>
<td>22</td>
<td>7–10</td>
<td>3–5</td>
</tr>
<tr>
<td>% Cholesteryl esters(^b)</td>
<td>3–5</td>
<td>10–15</td>
<td>30</td>
<td>35–40</td>
<td>12</td>
</tr>
<tr>
<td>Major apolipoproteins</td>
<td>A-I, A-II, B-48, C-I, C-II, C-III, E</td>
<td>B-100, C-I, C-II, C-III, E</td>
<td>B-100, C-I, C-II, C-III, E</td>
<td>B-100</td>
<td>A-I, A-II, C-I, C-II, C-III, D, E</td>
</tr>
</tbody>
</table>

\(^a\)Surface components  
\(^b\)Core lipids.
Apolipoproteins coat lipoprotein surface

- Apolipoprotein = apoprotein
- Nine different types
- LDL contains apo B-100
  4536 Aa, monomer, one of the largest proteins
  each LDL particle contains only 1 apo B-100
  α-helical
  amphipathic faces -> helical wheel
Cells take up LDL by receptor-mediated endocytosis.
2) Fatty acid oxidation

- **Hormone sensitive lipase** releases fatty acids from intracellular TAG stores

- **Lipoprotein lipases** releases fatty acids from lipoproteins into blood stream

- Fatty acids enter blood stream, kept soluble by binding to albumin, $\sim 10^{-6} \text{M} \rightarrow 2\text{mM}$

- But **analbuminemia** is not lethal

- Intracellular catabolism of fatty acids to produce energy
\( \beta \)-oxidation of fatty acids

- degradation of fatty acid through oxidation of \( C_\beta = \beta \)-oxidation

- mitochondria, matrix

- FA need to cross 2 membranes to reach matrix

- not as CoAs but as acyl-carnitine

- CPT-I, cytosol; CPT-II, matrix

- separate pools of mitoch/cytosol.
  - CoAs; ATPs; NAD\(^+\)
Franz Knoop’s classic experiment indicating that fatty acids are metabolically oxidized at their $\beta$-carbon atom

- Phenyl-labeled even- or odd-numbered fatty acids
- Feed to dogs -> what product appears in urine?

**Fatty acid fed**

Odd-chain fatty acid: \( \text{Phenyl-} \stackrel{(n+1)\ C_2}{\longrightarrow} \text{Benzoic acid} \rightarrow \text{Hippuric acid} \)

Even-chain fatty acid: \( \text{Phenyl-} \stackrel{(n+1)\ C_2}{\longrightarrow} \text{Phenylacetic acid} \rightarrow \text{Phenylaceturic acid} \)
A) Fatty acid activation catalyzed by acyl-CoA synthetase

- Fatty acids need almost always be activated to Acyl-CoAs for subsequent enzymatic reaction
- Activation by acyl-CoA synthetases via acyladenylate intermediate

\[
\text{FA} + \text{CoA} + \text{ATP} \leftrightarrow \text{Acyl-CoA} + \text{AMP} + \text{PP}_i
\]

High-energy thioester bond
Mechanism of fatty acid activation catalyzed by acyl-CoA synthetase

1) Activation of acyl chains to acyl-CoAs in cytosol
2) Requires ATP -> acyl-adenylate intermediated
3) Transesterification to CoA
4) Driven by inorganic pyrophosphatase $\text{PP}_i \rightarrow \text{H}_2\text{O} + 2\text{P}_i$
5) $^{18}\text{O}$-labels AMP and Acyl-CoA
B) Transport of fatty acids into the mitochondrial matrix

**as:** acyl-carnitine, through carnitine carrier protein IMM

Energy neutral, no ATP required, but highly regulated !!!

![Diagram showing the transport of fatty acids into the mitochondrial matrix.](image)
Acetyl-CoA + Carnitine → Acyl-Carnitine + CoA

Carnitine Palmitoyltransferase (CPT) catalyzes the transfer of an acetyl group from CoA to carnitine. This reaction is an important step in the regulation of mitochondrial fatty acid metabolism. The reaction is reversible, and the equilibrium is close to 1, indicating that the reaction is highly efficient.

\[
(CH_3)_3N^+ - CH_2 - CH - CH_2 - COO^- + R - C - SCoA \rightleftharpoons \text{Acyl-carnitine} + H - SCoA
\]
Transport of fatty acids across the mitochondrial double membrane
C) β-oxidation

- Chemically resembles the cytric acid cycle: Decarboxylation of succinate via fumarate and malate to oxaloacetate.
β-oxidation, 4 steps

1. Formation of trans-α,β double bond, by FAD-dependent acyl-CoA dehydrogenase (AD)

2. Hydration of the double bonds by enoyl-CoA hydratase (EH) to form 3-L-hydroxyacyl-CoA

3. NAD⁺-dependent dehydrogenation by 3-L-hydroxyacyl-CoA dehydrogenase (HAD) to form β-ketoacyl-CoA

4. Cα-Cβ cleavage by β-ketoacyl-CoA thiolase (KT, thiolase) → acetyl-CoA and C2 shortened acyl-CoA
The β-oxidation pathway of fatty acyl-CoA

Long chain versions of EH, HAD and KT in α₄β₄ ocatmeric protein, mitochondrial trifunctional protein -> channeling, no detectable intermediates.
trans-Δ²-Enoyl-CoA

2. H₂O
enzoyl-CoA hydratase (EH)

3. H
3-L-Hydroxyacyl-CoA

3. NAD⁺
3-L-hydroxyacyl-CoA dehydrogenase (HAD)
NADH + H⁺

4. CoASH
β-Ketoacyl-CoA thiolase (KT)

Fatty acyl-CoA (2 C atoms shorter) + Acetyl-CoA
Acyl-CoA dehydrogenases

• **1st step**: acyl-CoA dehydrogenases (AD)
  • mitos contain 4 such dehydrogenases with different chain length specificities
• VLCAD (C12-C18), LCAD (C8-C12)
• MCAD (C6-C10)
• SCAD (C4-C6)

• MCAD deficiency linked to 10% of cases of sudden infant death syndrome (SIDS): imbalance between glucose and fatty acid oxidation

• Reoxidation of FADH$_2$ by mitochondrial electron transport chain $\rightarrow$ ATP

MCAD, homo-tetramer FAD green
Mitochondrial trifunctional protein

2-enoyl-CoAs are further processed by chain length-specific:
- Enoyl-CoA hydratase (EHs)
- Hydroxyacyl-CoA dehydrogenase (HADs)
- $\beta$-ketoacyl-CoA thiolase (KTs)

- Long chain version contained $\alpha_4\beta_4$ octameric protein = **mitochondrial trifunctional protein**
  - $\alpha$ chain contains LCEH and LCHAD
  - $\beta$ chain LCKT
  (multifunctional protein, more than one enzyme on pp)

Multienzyme complex
Channeling of intermediates
Mechanism of action of β-ketoacyl-CoA thiolase

- Final step in β-oxidation
- Via an enzyme thioester bound intermediate to the substrates oxidized β carbon, displaced by CoA
Energy balance of $\beta$-oxidation

for C16 palmitic acid: 7 rounds of $\beta$-oxidation
$\rightarrow$ 8 x acetyl-CoA

Each round of $\beta$-oxidation produces:
- 1 NADH $\rightarrow$ 3 ATP
- 1 FADH$_2$ $\rightarrow$ 2 ATP
- 1 acetyl-CoA $\rightarrow$ TCA (1 GTP, 3 NADH, 1 FADH$_2$) (respiration only !)

OVERALL NET YIELD: **106 ATP per C16**
Special cases of $\beta$-oxidation

**Unsaturated fatty acids**
- mono, $\Delta 9$ (odd)
- poly, $\Delta 9, \Delta 12$ (odd, even)
  $\rightarrow$ isomerization, reduction

**Odd chain length fatty acids**
$\rightarrow$ propionyl-CoA in the last cycle

**Very long-chain fatty acids ($> C22$ atoms)**
$\rightarrow$ first $\beta$-oxidation in peroxisomes

**Branched chain fatty acids**
- chlorophyll’s phytanic acid
  $\rightarrow \alpha$-oxidation, formyl-CoA + propionyl-CoA
D) Oxidation of unsaturated fatty acids

Structures of two common unsaturated fatty acids, Usually, \textit{cis} double bond at C9
Additional double bond in C3 intervals, i.e. next at C12
-> odd, even numbered C atoms

Problems for $\beta$-oxidation

\textbf{Oleic acid}  
(9-	extit{cis}-Octadeccenoic acid)

\textbf{Linoleic acid}  
(9,12-	extit{cis}-Octadecadienoic acid)
Problems for β-oxidation of unsaturated fatty acids

1) Generation of a β, γ double bond
2) A Δ4 double bond inhibits hydratase action
3) Isomerization of 2,5-enoyl-CoA by 3,2-enoyl-CoA isomerase
Problem 1: Generation of a $\beta$, $\gamma$ double bond

No substrate for hydroxylase
Problem 1:
Generation of a $\beta, \gamma$ double bond

No substrate for hydroxylase
Problem 1: 
$\beta,\gamma$ double bond

$\text{enoyl-CoA isomerase}$

$\text{NAD}^+ + \text{FAD} + \text{CoASH}$
$\text{NADH} + \text{FADH}_2 + \text{Acetyl-CoA}$

one round of $\beta$ oxidation + the first oxidation of the next round

Problem 2: 
$\Delta^+ \text{ double bond}$

$\text{NADPH + H}^+$
$\text{NADP}^+$

2,4-dienoyl-CoA reductase (mammalian)

2,4-dienoyl-CoA reductase (E. coli)

3,2-enoyl-CoA isomerase (mammalian)

Continuation of $\beta$ oxidation
No substrate for hydroxylase

Stability of DB
E) Oxidation of odd chain fatty acids yields propionyl-CoA

- Most naturally FA are even numbered
- Odd numbered FA are rare, some plants and marine organisms
- Final round of $\beta$-oxidation yields propionyl-CoA
- Propionyl-CoA is converted to succinyl-CoA $\rightarrow$ TCA
- Propionate is also produced by oxidation of Ile, Val, Met
- Ruminant animals, most caloric intake from acetate and propionate produced by microbial fermentation of carbohydrates in their stomach
Propionyl-CoA → succinyl-CoA

3-step reaction:

1) Propionyl-CoA carboxylase, tetrameric enzyme with biotin as prosthetic group, C3→C4

2) Methylmalonyl-CoA racemase

3) Methylmalonyl-CoA mutase, B12 containing (cobalamin)
The rearrangement catalyzed by methylmalonyl-CoA mutase

Vit B12-dependent (cobalamin)

Highly stereospecific (R-methylmalonyl-CoA) → racemase
1. Heme-like corrin ring
2. 4 pyrrol N coordinate 6 fold coordinated Co
3. 5,6 coordination by dimethylbenzimidazole and deoxyadenosyl (C-Co bond !)
4. In carbon-carbon rearrangements
5. Methyl group transfer
6. About 12 known B12-dependent enzymes
7. Only 2 in mammals
   a. Methylmalonyl mutase, homolytic cleavage, free radical mechanism
   b. Methionine synthase
8. B12 acts as a reversible free radical generator, hydrogen rearrangement or methyl group transfer by homolytic cleavage
X-Ray structure of *P. shermanii* methylmalonyl-CoA mutase in complex with 2-carboxypropyl-CoA and AdoCbl

\[ \alpha/\beta \]-barrel class of enzymes
Proposed mechanism of methylmalonyl-CoA mutase
Vit B12 deficiency

Pernicious anemia
- in elderly
- decreased number of red blood cells
- treated by daily consumption of raw liver (1926) -> (1948)
- only few bacteria synthesize B12, plants and mammals not
- human obtain it from meat
- Vit. B12 is specifically bound in intestine by intrinsic factor
- complex absorbed in intestinal mucosa -> blood
- bound to transcobalamins in blood for uptake by tissue
- not usually a dietary disease but result from insufficient secretion of intrinsic factor
The fate of Succinyl-CoA

1) Succinyl-CoA is not consumed in TCA cycle but has a catalytic function
2) To consume it, it must first be converted to pyruvate or acetyl-CoA
   - Conversion to malate (TCA)
   - Export of malate to cytosol, if conc. are high
   - Conversion to pyruvate by malic enzyme
F) Peroxisomal $\beta$ oxidation

- $\beta$-oxidation occurs both in mitochondria and in peroxisomes
- **Peroxisomes:** Shortening of very-long chain fatty acids (VLCFA) for subsequent transport and oxidation in mitochondria
  - ALD protein to transport VLCFA into peroxisomes, no carnitine required, VLCFA-CoA synthetase
  - **X-adrenoleukodystrophy caused by defects in ALD,** lethal in young boys, 13% reduced efficiency of lignoceric acid (C24:0) to lignoceryl-CoA conversion
  - first step in perox. oxid. **Acyl-CoA oxidase** generates $\text{H}_2\text{O}_2$ (peroxide) $\rightarrow$ name! Catalase
  - carnitine for transport of chain shortened FAs out of peroxisomes and into mito.
Peroxisomal β-oxidation

First step:

\[ \text{Fatty acyl-CoA} + \text{O}_2 \rightarrow \text{enoyl-CoA} + \text{H}_2\text{O}_2 \]

catalyzed by acyl-CoA oxidase

FAD dependent but direct transfer of electrons to \( \text{O}_2 \rightarrow \text{H}_2\text{O}_2 \)
Pathway of $\alpha$ oxidation of branched chain fatty acids

- $\beta$-oxidation is blocked by methyl group at $C_\beta$

- Phytanic acid, breakdown product of Chlorophyll’s phytol side chain
- Degraded by $\alpha$-oxidation
- generates formyl-CoA
- and propionyl-CoA
- C-end will give 2-methyl-propionyl-CoA
- Refsum disease/phytanic acid storage d.
- omega oxidation in the ER, Cyt P450
3) Ketone bodies

- Fate of acetyl-CoA generated by $\beta$-oxidation:
  1. TCA cycle
  2. Ketogenesis in liver mitochondria

- Ketone bodies, fuel for peripheral tissue (brain !)
- where they are again converted into acetyl-CoA
- water soluble equivalent of fatty acids
Ketogenesis

3 step reaction:
1. Condensation of 2 acetyl-CoA -> acetoacetyl-CoA (reversal of thiolase rxt)
2. Addition of third acetyl-CoA
3. Cleavage by HMG-CoA lyase

Ketosis:
Spontaneous decarboxylation of acetoacetate to CO₂ and acetone breath (more fuel than used)
The metabolic conversion of ketone bodies to acetyl-CoA in the periphery

Liver lacks ketoacyl-CoA transferase -> export of acetoacetyl/hydroxybutyrate
4) Fatty acid Synthesis

Synthesis of FA through condensation of C2 \((C3-CO_2)\) units -> reversal of \(\beta\)-oxidation

Cytosolic, NADPH <-> mitochondrial, FAD, NAD
Difference in stereochemistry
C3 unit for growth (malonyl-CoA) <-> C2 for oxidation (acetyl-CoA)

Growing chain esterified to acyl-carrier protein (ACP)
Esterified to phosphopantetheine group as in CoA which itself is bound to a Ser on ACP

ACP synthase transfers phosphopantetheine to apo-ACP to form a holo-ACP
A comparison of fatty acid β oxidation and fatty acid biosynthesis

**β Oxidation**
- Occurs in mitochondrion
- CoA is acyl group carrier
- FAD is electron acceptor

1. Fatty acyl-CoA (C_{n+2})
2. Enoyl-CoA
3. 3-β-Hydroxyacyl-CoA
4. β-Ketoacyl-CoA
5. Fatty acyl-CoA (C_{n})

**Biosynthesis**
- Occurs in cytoplasm
- ACP is acyl group carrier
- NADPH is electron donor

1. Fatty acyl-ACP (C_{n+2})
2. 3-β-Hydroxyacyl-ACP
3. β-Ketoacyl-ACP
4. β-Ketoacyl-ACP
5. Fatty acyl-ACP (C_{n})
A) Mitochondrial acetyl-CoA must be transported into cytosol

- **Acetyl-CoA**: produced by pyruvate dehydrogenase, \( \beta \)-oxidation in mitochondria

- Acetyl-CoA enters the cytosol in form of **citrate** via the tricarboxylate transporter

- **In the cytosol:**
  - Citrate + CoA + ATP <-> acetyl-CoA + OXA + ADP + Pi (citrate lyase)
  - citrate export balanced by anion import (malate, pyruvate, or \( P_i \))
B) Acetyl-CoA carboxylase produces malonyl-CoA

- Catalyzes first and committed step of FA synthesis
- Biotin-dependent (see propionyl-CoA carboxylase)
- Stimulated by citrate!
- Hormonally regulated: Glucagon → cAMP up → PKA → ACC is phosphorylated (inactivated) → activated by insulin
- Mammals two isoforms:
  - \(\alpha\)-ACC, adipose tissue
  - \(\beta\)-ACC, tissue that beta-oxidize FA, heart muscle, regulates \(\beta\)-ox. as malonyl-CoA inhibits CPT-I
Biotin-dependent carboxylation reactions

1) **Acetyl-CoA carboxylase** $\rightarrow$ **malonyl-CoA**
   (fatty acid synthesis)

2) **Propionyl-CoA carboxylase** $\rightarrow$ **methylmalonyl-CoA**
   ($\beta$-oxidations of odd chain fatty acids)

3) **Pyruvate carboxylase** $\rightarrow$ **oxalacetate**
   (TCA cycle, gluconeogenesis)

Always:

1) **Carboxylation of biotin by bicarbonate**, ATP requiring

2) **Stereospecific transfer of carboxyl group**
**Ping-Pong Mechanism**

1. **ATP** + **HCO\textsubscript{3}** → **Biotinyl-enzyme**

2. **ADP** + **P\textsubscript{i}** → **Carboxybiotinyl-enzyme**

**Malonyl-CoA**

- **(S)-Methylmalonyl-CoA** + **Biotinyl-enzyme**
Association of acetyl-CoA carboxylase protomers

- Multifunctional protein in eukaryotes (1 polypeptide chain)
- Composed of 3 proteins in bacteria:
  - Biotin carboxylase
  - Transcarboxylase
  - Biotin carboxyl-carrier
- Polymerizes upon activation
C) Fatty acid synthase catalyzes seven reactions

- Synthesis of FA from acetyl-CoA (starter) and malonyl-CoA (elongation) requires 7 enzymatic reactions,
- 7 proteins in E. coli + ACP
- $\alpha_6\beta_6$ complex in yeast (2500 kD)
- homodimer in mammals, 272 kD

EM-based image of the human FAS dimer as viewed along its 2-fold axis, each monomer has 4 50 Å diameter lobs -> functional domains antiparallel orientation
The animal fatty acid synthase (FAS)

Multifunctional protein with 7 catalytic activities
Head to tail interaction of monomer in the dimer (KS close to ACP)

Two monomers operate in concert (534 kD).

Ketoacyl ACP synthase  β-hydroxyacyl-ACP dehydratase  β-ketoacyl-ACP reductase
Malonyl/acetyl-CoA-ACP transacylase  Enoyl-ACP reductase  Palmitoyl thioesterase
The phosphopantetheine group in acyl-carrier protein (ACP) and in CoA

Phosphopantetheine prosthetic group of ACP

Phosphopantetheine group of CoA
The mechanism of carbon–carbon bond formation in fatty acid biosynthesis

$\text{CO}_2$ that has been incorporated into malonyl-CoA is not found in the final Fatty Acid!

But makes the reaction irreversible!
Acetoacetyl-ACP

\[ \text{H}^+ + \text{NADPH} \rightarrow \beta\text{-ketoacyl-ACP reductase (KR)} \]

\[ \text{NADP}^+ \rightarrow \]

\[ \text{d-\beta-Hydroxybutyryl-ACP} \]

\[ \text{H}_2\text{O} \rightarrow \beta\text{-hydroxyacyl-ACP dehydrase (DH)} \]

\[ \alpha,\beta\text{-trans-Butenoyl-ACP} \]

\[ \text{H}^+ + \text{NADPH} \rightarrow \text{enoyl-ACP reductase (ER)} \]

\[ \text{NADP}^+ \rightarrow \]

\[ \text{Butyryl-ACP} \]

recycle Reactions 2a–5 six more times

after 7 reaction cycles

\[ \text{Palmitoyl-ACP} \]

\[ \text{H}_2\text{O} \rightarrow \text{palmitoyl thioesterase (TE)} \]

\[ \text{Palmitate} + \text{H}^- \rightarrow \text{SACP} \]
Recycle Reactions 2a–5 six more times after 7 reaction cycles.

\[
\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{C}^-\text{SACP} \rightarrow \text{Butyryl-ACP}
\]

\[
\text{CH}_3\text{CH}_2\text{(CH}_2\text{)}_{13}\text{C}^-\text{SACP} \rightarrow \text{Palmitoyl-ACP}
\]

\[
\text{H}_2\text{O} \xrightarrow{6} \text{palmitoyl thioesterase (TE)} \rightarrow \text{Palmitate} + \text{H}^-\text{SACP}
\]
Triclosan: An inhibitor of bacterial fatty acid synthesis

- In cosmetics, toothpastes, toys etc
- As antibacterial agent
- inhibits bacterial enoyl-ACP reductase
- resistance is developing....

![Triclosan](image.png)
D) Fatty acids may be elongated and desaturated

Elongation at carboxy terminus:
- mitochondria (reversal of β-ox)
- ER (malonyl-CoA)
FA desaturation

Properties:
- Cis, Δ9 first, not conjugated
- membrane-bound, nonheme iron enzymes, cyt b5-dependent
- mammals front end desaturation (Δ9, 6, 5/4)
- essential FA, linoleic (C18:2n-6, Δ9,12), linolenic (C18:3n-3, Δ9,12,15)
- some made by combination of desaturation and elongation
- PUFAs, fish oil, n-3, n-6 (omega)
- vision, cognitive functions

The electron-transfer reactions mediated by the Δ9-fatty acyl-CoA desaturase complex
E) Fatty acids are esterified to form triacylglycerol

TAG are synthesized from fatty acyl-CoAs and *glycerol-3-phosphate* or *dihydroxyacetone phosphate*

- Glycerol-3-phosphate acyltransferase in ER and mitochondria
- DHP acyltransferase in ER and peroxisomes

Glyceroneogenesis in liver
Partial gluconeogenesis from oxalacetate
phosphate

1-acylglycerol-3-phosphate acyltransferase

H-SCoA

R-C-SCoA

CH₂-O-C-R

CH₂-O-PO₃⁻

Phosphatidic acid

Phosphatidic acid phosphatase

P₁

R-C-SCoA H-SCoA

2-monoacylglycerol acyltransferase

R'-C-O-C-H

CH₂-OH

2-Monoacylglycerol (from intestinal digestion)

R'-C-O-C-H

CH₂-OH

1,2-Diacylglycerol (DAG)

R'-C-SCoA H-SCoA

diacylglycerol acyltransferase

H-SCoA

R'-C-SCoA

CH₂-O-C-R

CH₂-O-C-R

Triacylglycerol
Summary of lipid metabolism

Triacylglycerols

Membrane lipids ↔ Fatty acids

fatty acid synthesis

NADPH, ATP

β oxidation

FADH$_2$, NADH

Cholesterol

Acetyl-CoA

Ketone bodies

Citric acid cycle

oxidative phosphorylation

NADH, FADH$_2$, GTP → ATP
5) Regulation of fatty acid metabolism

Differences in energy needs:
- between resting and activated muscle 100x
- feed <-> fasting

- Breakdown of glycogen and fatty acids concern the whole organism
- organs and tissues connected by blood stream, coordination

- Blood glucose levels sensed by pancreatic $\alpha$ cells, glucose down -> secrete glucagon -> glycogen degradation,
- $\beta$ cells, glucose up -> insulin -> glucose uptake, FS synthesis

- These hormones also control fatty acid synthesis <-> $\beta$ oxidation
Two levels of metabolic control

Short term regulation
regulates catalytic activities of key enzymes in minutes or less:
substrate availability
allosteric interactions
Covalent modification
-> ACC (activated by citrate, inhibited by palmitoyl-CoA, inactivated by phosphorylation)

Long term regulation
amount of enzyme present, within hours or days
-> ACC
6) Phospholipid and glycerolipid metabolism:

The glycerolipids and sphingolipids

Glycerolipid

\[
\begin{align*}
\text{R}_2 - C - O - C - H & \quad \text{O} \\
\text{CH}_2 - O - C - R_1 & \quad \text{R}_2 - C - O - C - H \\
\text{CH}_2 - O - X & \quad \text{OH} \\
\end{align*}
\]

Sphingolipid

\[
\begin{align*}
\text{R}_2 - C - O - C - H & \quad \text{O} \\
\text{CH}_2 - O - C - R_1 & \quad \text{R}_2 - C - N - H - C - H - C - H - H \\
\text{CH}_2 - O - X & \quad (\text{CH}_2)_{12} - \text{CH}_3 \\
\end{align*}
\]

\[X = H \quad \text{1,2-Diacylglycerol} \quad \text{N-Acylsphingosine (ceramide)}
\]

\[X = \text{Carbohydrate} \quad \text{Glyceroglycolipid} \quad \text{Sphingoglycolipid (glycosphingolipid)}
\]

\[X = \text{Phosphate ester} \quad \text{Glycerophospholipid} \quad \text{Sphingophospholipid}\]
Membrane lipids

Amphipathic: hydrophobic tail / hydrophilic head
- glycerol, 1,2-diacyl-sn-glycerol
- N-acylsphingosine (ceramide)
- Head:
  - phosphate ester
  - carbohydrate

- 2 categories of phospholipids:
  Glycerophospholipids, sphingophospholipids

- 2 categories of glycolipids
  Glyceroglycolipids, sphingoglycolipids/glycosphingolipids
A) Glycerophospholipids are built from intermediates of Triacylglycerol biosynthesis

- sn-1: prevalence saturated FA
- sn-2: prevalence unsaturated FA

Biosynthesis of diacylglycerophospholipids
  - from DAG and PA as TAG synthesis

Head group addition:
  - **PC/PE**
    - P-activated Etn or Cho
    - → CDP-activated Etn or Chol
    - → transfer on DAG
  - **PS**, head-group exchange on PE with Serine
  - **PI/PG**, CDP-DAG
The biosynthesis of phosphatidylethanolamine and phosphatidylcholine

- DAG and CDP-ethn or CDP-chol
- Methylation pathway in the liver
  PE $\rightarrow$ PC, SAM-dependent
CTP:phosphoethanolamine cytidyltransferase
or CTP:phosphocholine cytidyltransferase

\[
\text{Cytidine} \rightarrow \text{CTP}
\]

\[
\text{CTP} \rightarrow \text{PP}_i
\]

\[
\text{R'} = H \quad \text{CDP-ethanolamine}
\]
\[
\text{R'} = \text{CH}_3 \quad \text{CDP-choline}
\]

CDP-ethanolamine:1,2-diacylglycerol phosphoethanolamine transferase
or CDP-choline:1,2-diacylglycerol phosphocholine transferase

\[
\text{1,2-Diacylglycerol} \rightarrow \text{CMP}
\]

\[
\text{R}_2 - \text{C} - \text{O} - \text{C} - \text{H}
\]
\[
\text{CH}_2 - \text{O} - \text{C} - \text{R}_1
\]
\[
\text{O} - \text{CH}_2 - \text{O} - \text{C} - \text{R}_1
\]

\[
\text{R'} = H \quad \text{Phosphatidylethanolamine}
\]
\[
\text{R'} = \text{CH}_3 \quad \text{Phosphatidylcholine (lecithin)}
\]
Phosphatidylserine synthesis

Head group exchange on PE with Serine

Phosphatidylethanolamine + Serine

phosphatidylethanolamine: serine transferase

Phosphatidylserine
The biosynthesis of phosphatidylinositol and phosphatidylglycerol

**CDP-DAG** activated DAG
+ inositol or glycerol-3P
= PI or PG
The formation of cardiolipin

Mitochondrial phospholipid
2x PG = CL + Glycerol

Phosphatidylglycerol
Cardiolipin
FA Remodeling

Tissue and cell-type specific introduction of defined FA into lipids

Examples:
- 80% of brain PI contains C18:0 in sn-1 and C20:4 in sn-2
- 40% of lung PC has C16:0 in both positions, surfactant
Plasmalogens

Around 20% of mammalian PLs are plasmalogens
  - Nervous tissue
  - Mainly PEs

1. Plasmalogens: vinyl ether linkage in C1
2. Alkylacylglycerophospholipids: ether linkage

![Chemical structures]

A plasmalogen

An alkylacyl-glycerophospholipid
B) Sphingolipids

1. Cover the external surface of the plasma membrane, biosynthesis in ER/Golgi lumen

2. **Sphingomyelin** is major phosphosphingolipid, phosphocholine head group, not from CDP-choline but from PC

3. **Sphingoglycolipids**
   1. Cerebrosides, ceramide monosaccharides
   2. Sulfatides, ceramide monosaccharides sulfates
   3. Globosides, neutral ceramide oligosaccharides
   4. Gangliosides, acidic, sialic acid-containing ceramide oligosaccharides
The biosynthesis of ceramide

1) Serine + palmitoyl-CoA = KS
2) Reduction of KS to sphinganine (LCB)
3) LCB + Acyl-CoA = ceramide (DHC)
4) Oxidation of DHC to Cer
The synthesis of sphingomyelin from \( N \)-acylsphingosine and phosphatidylcholine

\[
\text{Ceramide (N-acylsphingosine)} + \text{Phosphatidylcholine} \rightarrow \text{Diacylglycerol} \rightarrow \text{Sphingomyelin}
\]

- PC is head-group donor to convert Cer to SM
Principal classes of sphingoglycolipids

**Cerebrosides**
- **Glucocerebroside**
- **Galactocerebroside**

**Sulfatide**

**Globosides**
- **Lactosyl ceramide**
- **Trihexosyl ceramide**

**Globoside**

**Gangliosides**
- **G\textsubscript{M3}**
- **G\textsubscript{M2}**
- **G\textsubscript{M1}**

= glucose

= N-acetylglactosamine

= galactose

= ceramide

NANA = N-acetylneuraminic acid (sialic acid)
Sphingoglycolipid degradation and lipid storage disease

- Degraded in lysosomes by series of enzyme-mediated hydrolytic steps
- Catalyzed at lipid-water interface by soluble enzymes
- Aid of SAPS, sphingolipid activator proteins
- $GM_2$-activator-$GM_2$ complex binds hexosaminidase A
  that hydrolyzes N-acetylgalactosamine from $GM_2$

- Enzymatic defect leads to sphingolipid storage disease, e.g.,
  **Tay-Sachs disease**, deficiency in hexosaminidase A,
  neuronal accumulation of $GM_2$ as shell-like inclusions,
  In utero diagnosis possible with fluorescent substrate

- Substrate deprivation therapy, inhibition of glucosyl-
eramide synthase
Cytoplasmic membranous body in a neuron affected by Tay-Sachs disease

Most common SL storage disease
Hexosaminidase deficiency
Cytoplasmic membrane bodies in neurons
Model for $G_{M2}$-activator protein-stimulated hydrolysis of ganglioside $G_{M2}$ by hexosaminidase
The breakdown of sphingolipids by lysosomal enzymes
# Sphingolipid Storage Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme Deficiency</th>
<th>Principal Storage Substance</th>
<th>Major Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>G\textsubscript{M1} Gangliosidosis</td>
<td>G\textsubscript{M1} (\beta)-galactosidase</td>
<td>Ganglioside G\textsubscript{M1}</td>
<td>Mental retardation, liver enlargement, skeletal involvement, death by age 2</td>
</tr>
<tr>
<td>Tay–Sachs disease</td>
<td>Hexosaminidase A</td>
<td>Ganglioside G\textsubscript{M2}</td>
<td>Mental retardation, blindness, death by age 3</td>
</tr>
<tr>
<td>Fabry’s disease</td>
<td>(\alpha)-Galactosidase A</td>
<td>Trihexosylyceramide</td>
<td>Skin rash, kidney failure, pain in lower extremities</td>
</tr>
<tr>
<td>Sandhoff’s disease</td>
<td>Hexosaminidases A and B</td>
<td>Ganglioside G\textsubscript{M2} and globoside</td>
<td>Similar to Tay–Sachs disease but more rapidly progressing</td>
</tr>
<tr>
<td>Gaucher’s disease</td>
<td>Glucocerebrosidase</td>
<td>Glucocerebroside</td>
<td>Liver and spleen enlargement, erosion of long bones, mental retardation in infantile form only</td>
</tr>
<tr>
<td>Niemann–Pick disease</td>
<td>Sphingomyelinase</td>
<td>Sphingomyelin</td>
<td>Liver and spleen enlargement, mental retardation</td>
</tr>
<tr>
<td>Farber’s lipogranulomatosis</td>
<td>Ceramidase</td>
<td>Ceramide</td>
<td>Painful and progressively deformed joints, skin nodules, death within a few years</td>
</tr>
<tr>
<td>Krabbe’s disease</td>
<td>Galactocerebrosidase</td>
<td>Deacylated galactocerebroside</td>
<td>Loss of myelin, mental retardation, death by age 2</td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td>Arylsulfatase A</td>
<td>Sulfatide</td>
<td>Mental retardation, death in first decade</td>
</tr>
<tr>
<td>(Sulfatide lipidosis)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C) C20 fatty acids are the precursors of Prostaglandins (PGs)

- 1930, Ulf von Euler: human semen extract stimulates uterus contraction and lower blood pressure
- Thought to originate in prostatic -> name
- Mid 50s, isolated from body fluids in ether extract (PGE)
- Made by all cells except RBC
Eicosanoid metabolism: Prostaglandins, prostacyclins, thromboxanes, leukotriens, and lipoxins

Collectively: **eicosanoids**, C20 compounds
- profound physiological effects at very low conc.
- hormone-like but paracrine
- bind to G-coupled receptors, affect cAMP
- signal as hormones do
- arachidonic acid C20:4

What you inhibit by **aspirin** !!
NSAIDs, nonsteroidal anti-inflammatory drugs

Whose action you indirectly inhibit by **cortisol** !!
Eicosanoids

Mediate:
1) inflammation
2) production of pain and fever
3) regulate blood pressure
4) induction of blood clotting
5) reproductive functions
6) sleep/wake cycle
7) Egress of T lymphocytes
Prostaglandin structures. (a) The carbon skeleton of prostanoic acid, the prostaglandin parent compound.

Cyclopentane ring
Synthesized from arachidonic acid, C20:4, Δ5,8,11,14 (ω-6 FA)
Prostaglandin structures. (c) Structures of prostaglandins $E_1$, $E_2$, and $F_{2\alpha}$ (the first prostaglandins to be identified)
Arachidonic acid is the precursor to PGs

- Arachidonic acid: C20:4, n-6, Δ5,8,11,14

- AA is synthesized from the essential linoleic acid, C18:3, Δ6,9,12 by elongation and desaturation

- AA is phospholipid bound (sn2, PI) and released upon stimuli by:
  1) phospholipase A2
  2) phospholipase C → DAG + P-Ins → PA (DAG kinase) → AA (PLA2)
  3) DAG hydrolysis by DAG lipase

- Corticosteroids indirectly inhibit PG signaling !!
  anti-inflammatory
Release of arachidonic acid by phospholipid hydrolysis

phospholipase A₂

H₃₁C₁₉—C

Arachidonoyl group

O

CH₂—O—C—R₁

O

CH₂—O—P—X

phospholipase C

X =

Inositol
Pathways of arachidonic acid liberation from phospholipids

Phospholipid (phosphatidylinositol)

- phospholipase A₂
- phospholipase C

Lysophospholipid + Arachidonic acid

- diacylglycerol kinase
- diacylglycerol lipase

1,2-Diacylglycerol (DAG)

- Phosphatidic acid
  - phospholipase A₂

- Monoacylglycerol + Arachidonic acid

Lysophosphatidic acid + Arachidonic acid
The cyclic pathway of arachidonic acid metabolism
The reactions catalyzed by PGH synthase (PGHS)

- **PGHS** catalyzes first step in the cyclic pathway
- Cyclooctynase (COX) + peroxidase activity
- Heme activates Tyr radical
- Target of aspirin
- Monotopic membrane protein (see squalene-hopene cyclase)
X-Ray structure of PGH synthase (PGHS) from sheep seminal vesicles in complex with the NSAID flurbiprofen

Homodimeric monotopic ER membrane protein

Heme
Fluriprofen
Active side Tyr

ER lumen

Courtesy of Michael Garavito, Michigan State University
ASPIRIN

- Acetylsalicylic acid
- Inhibits cyclooxygenase activity of PGHS
- Acetylates Ser 530
- Flurbiprofen blocks channel
- Low dose of aspirin reduce heart-attack risk, inhibits platelet aggregation (enucleated cells, 10 days lifetime, cannot resynthesize enzyme)
Inactivation of PGH synthase by aspirin
Some nonsteroidal anti-inflammatory drugs (NSAIDs)

- Aspirin (acetylsalicylic acid)
- Ibuprofen
- Flurbiprofen
- Acetaminophen
- Indomethacin
- Naproxen
- Phenylbutazone
Vioxx

- 2 PGH synthase isoforms, **COX-1**, **COX-2**
  - **COX-1** is constitutively expressed in most tissues, including the gastrointestinal mucosa
  - **COX-2** only in certain tissues expressed in response to inflammatory stimuli

**Aspirin can induce gastrointestinal ulceration**

⇒ Search for selective **COX-2** inhibitors (coxibs) for long-term treatment, i.e. arthritis

**COX-3** may be the target of acetaminophen, widely used analgesic/antipyretic drug → treat pain & fever
COX-2 inhibitors

Rofecoxib (Vioxx)

Celecoxib (Celebrex)
Merck zahlt Vioxx-Opfern 4,85 Milliarden Dollar


27'000 Fälle
ESKIMOS

- Low risk of cardiovascular disease despite the fact that they eat a lot of fat, why?

- Are healthy because they eat fish, PUFAs, n-3, n-6

- Reduce cholesterol, leukotriene and PG levels
7) Cholesterol metabolism

- Vital constituent of cell membranes
- precursor to:
  - steroids
  - bile salts
- Cardiovascular disease, delicate balance!

All of cholesterol’s carbon atoms are derived from acetyl-CoA
Cholesterol is made by cyclization of squalene

Squalene from 6 isopren units (C30), polyisopren.
Part of a branched pathway that uses isoprenes
The branched pathway of isoprenoid metabolism in mammalian cells
HMG-CoA is a key cholesterol precursor

HMG-CoA is rate-limiting ER membrane enzyme, 888 Aa
1. Reduction to OH

Then:
1. Phosphorylation
2. Pyrophosphate
3. Decarboxylation/Dehydration

HMG-CoA reductase

\[ \text{HMG-CoA reductase} \xrightarrow{1} \text{2 NADPH} \]
\[ \xrightarrow{2} \text{2 NADP}^+ \]
\[ \xrightarrow{3} \text{CoA} \]

\[ \text{HMG-CoA} \]

\[ \xrightarrow{\text{HMG-CoA reductase}} \]
\[ \text{Mevalonate} \]
Action of pyrophosphomevalonate decarboxylase

5-Pyrophosphomevalonate

\[ \text{Pyrophosphomevalonate decarboxylase} \]

\[ \text{CO}_2 \]

\[ \text{Isopentenyl pyrophosphate} \]

\[ \text{ADP} \]
Formation of squalene from isopentenyl pyrophosphate and dimethylallyl pyrophosphate
Squalene synthase

- ER anchored
- Monomer single domain
The squalene epoxidase reaction

- Preparation for cyclization
- Oxygen required for cholesterol synthesis

\[
\begin{align*}
\text{Squalene} & \quad + \quad \text{O}_2 \\
& \quad \xrightarrow{\text{NADPH}} \quad \text{squalene epoxidase} \\
& \quad \xrightarrow{\text{NADP}^+} \\
\text{2,3-Oxidosqualene} & \quad + \quad \text{H}_2\text{O}
\end{align*}
\]
The oxidosqualene cyclase reaction

**Lanosterol synthase**
Folding of oxidosqualene on the enzyme!

**Related reaction in bacteria:**
$O_2$-independent
Squalene-hopene cyclase
Squalene-hopene cyclase

α/α barrel

Hopene

Active as homodimer

μονοτοπικός
membrane protein
Squalene-hopene cyclase with its membrane-bound region yellow

Hydrophobic channel from active site to membrane

Courtesy of Georg Schulz, Institut für Organische Chemie und Biochemie, Freiburg im Breisgau, Germany
The 19-reaction conversion of lanosterol to cholesterol

- 19 steps
- Loss of 3 methyl groups
- C30 -> C27
- One oxidation
- 9 $O_2$ dependent
- ER localized enzymes

Lanosterol -> cholesterol
Cholesterol

Liver synthesized cholesterol is:
- converted to bile salts
- esterified to cholesteryl ester, ACAT
which are then packaged into lipoprotein complexes, VLDL
and taken up by the tissue by LDL receptor mediated endocytosis

Mammalian cells thus have 2 ways to acquire cholesterol:
de novo synthesis or via LDL uptake

Dietary sterols are absorbed in small intestine and transported as chylomicrons in lymph to tissue/liver

HDL transports cholesterol from the peripheral tissue to the liver
LDL receptor-mediated endocytosis in mammalian cells
Regulation of cholesterol levels

Sterol Homeostasis:

1. HMG-CoA reductase, i.e. de novo synthesis
   short-term: competitive inhib., allosteric, cov. mod.
   long-term, rate of enzyme synthesis and degradation
   => SREBP PATHWAY !!

2. Regulation of LDL Receptor

3. Regulating esterification, ACAT
Cholesteryl esters

The transport and storage form of cholesterol

Cholesteryl ester
The SREBP Pathway

**SREBP**, membrane anchored transcription factor (1160 Aa)
480 Aa N-term, basic helix-loop-helix/leucine zipper dom. => binds SRE element
central 2 TMD, loop
590 Aa C-term regulatory domain

**SCAP**, integral membrane protein, ER, 1276 Aa
N-term 8 TMDs (730 Aa), Sterol-sensing domain
C-term, WD40 repeat => protein interaction (546 Aa)

1) Long term regulation of HMG-CoA reductase
2) Short term by phosphorylation via AMPK (see ACC1), P-form less active
3) LDL receptor
Model for the cholesterol-mediated proteolytic activation of SREBP

Little cholesterol in ER

Too much cholesterol in ER
Competitive inhibitors of HMG-CoA reductase used for the treatment of hypercholesterolemia

statins

Lovastatin (Mevacor)
Pravastatin (Pravachol)
Simvastatin (Zocor)

HMG-CoA
Mevalonate
COMBINATORIAL THERAPY

1) Anion exchanger, cholestyramine, reduced recycling of bile acids and uptake of dietary cholesterol => 15-20% drop

2) HMG-CoA inhibitor statins

Combined => 50-60% reduction of blood cholesterol levels
Control of plasma LDL production and uptake by liver LDL receptors. (a) Normal human subjects

(a) Normal

Liver

Normal LDL receptor function

VLDL

IDL

LDL

Capillary

lipoprotein lipase

Free fatty acids
Control of plasma LDL production and uptake by liver LDL receptors

(d) Overexpression of LDL receptor prevents diet-induced hypercholesterolemia
An atherosclerotic plaque in a coronary artery
The role of LDL and HDL in cholesterol metabolism

(a) Normal  (b) Familial hypercholesterolemia  (c) Tangier disease
Familial Hypercholesterolemia

- 1972, Brown and Goldstein, Nobel Price
- 1985, second for SREBP
- "you are as good as your next experiment"

A RECEPTOR-MEDIATED PATHWAY FOR CHOLESTEROL HOMEOSTASIS

Nobel lecture, 9 December, 1985
by
MICHAEL S. BROWN AND JOSEPH L. GOLDSTEIN

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