"Fatty Acid, Triacylglycerol, and Ketone Body Metabolism"

I. Overview

- Fatty acids exist:
    - Free in the body (i.e., nonesterified)
    - As fatty acyl esters in more complex molecules such as triacylglycerols (TAGs)
- Free fatty acids (FFA):
    - Occur in low levels in all tissues
    - Can be found in substantial amounts in plasma, particularly during fasting
- Plasma FFA:
    - Transported on serum albumin
    - In route from their point of origin:
        - TAG of adipose tissue
        - Circulating lipoproteins
    - Destination: site of consumption (most tissues)\
- FFA oxidation:
    - Can be oxidized by many tissues, particularly liver and muscle

- Purpose:
    - Provide energy
    - In the liver: provide substrate for ketone body synthesis

- Fatty acids are:
    - Structural components of membrane lipids:
        - Phospholipids
        - Glycolipids
    - Attached to certain proteins to enhance ability to associate with membranes
    - Precursors of hormone-like prostaglandins

- Esterified fatty acids (as TAG in white adipose tissue [WAT]):
    - Serve as the major energy reserve of the body

- Alterations in fatty acid metabolism:
    - Associated with obesity and diabetes

II. Fatty Acid Structure

- A fatty acid consists of:
  - Hydrophobic hydrocarbon chain
  - Terminal carboxyl group with a pKa of 4.8
- At physiologic pH:
  - Terminal carboxyl group (-COOH) ionizes to become -COO-
- Note:
  - When pH is above the pK, the deprotonated form predominates
- Amphipathic nature of fatty acids:
  - Due to anionic group's affinity for water
  - Fatty acid has both:
    - Hydrophilic region (-COO-)
    - Hydrophobic region (hydrocarbon chain)

- For long-chain-length fatty acids (LCFA):
    - Hydrophobic portion is predominant
    - These molecules are highly water insoluble
    - Must be transported in circulation in association with protein
- 90% of fatty acids in plasma:
    - Found in the form of fatty acid esters:
        - Primarily TAG
        - Cholesteryl esters
        - Phospholipids
    - Contained in circulating lipoprotein particles
- Free fatty acids (FFA):
    - Transported in circulation in association with albumin
    - Albumin: the most abundant protein in serum

A. Fatty Acid Saturation

- Fatty acid chains may:
  - Contain no double bonds → saturated
  - Contain one or more double bonds → mono- or polyunsaturated
- In humans:
  - The majority of fatty acids are saturated or monounsaturated
- When double bonds are present:
  - They are nearly always in the cis configuration
  - Cis double bonds cause the fatty acid to bend or kink at that position
- If a fatty acid has two or more double bonds:
  - They are always spaced at three-carbon intervals

- Note:
    - Addition of double bonds decreases the melting temperature (Tm) of a fatty acid
    - Increasing chain length increases the Tm
    - Membrane lipids typically contain long-chain fatty acids (LCFA)
        - Presence of double bonds helps maintain the fluid nature of those lipids

- In humans:
    - Fatty acids with even number of carbon atoms (16, 18, or 20) predominate
    - Longer fatty acids (>22 carbons) are found in the brain

- Carbon atom numbering:
    - Begins with the carbonyl carbon as carbon 1

- Notation system:
    - Number before the colon = number of carbon atoms in the chain
    - Numbers after the colon = positions of double bonds (relative to the carboxyl end)

- Example:
    - Arachidonic acid → 20:4(5,8,11,14)
        - 20 carbons long
        - 4 double bonds between:
            - Carbon 5–6
            - Carbon 8–9
            - Carbon 11–12
            - Carbon 14–15

- Note on carbon naming:
    - Carbon 2 = α-carbon
    - Carbon 3 = β-carbon
    - Carbon 4 = γ-carbon
    - Terminal methyl group carbon = ω-carbon, regardless of chain length

- Double bond position can also be referenced from the ω (methyl) end of the chain

- Arachidonic acid:
    - Called an ω-6 fatty acid
    - Terminal double bond is six bonds from the ω end
    - Equivalent designation: n-6

- Other examples:
    - Linoleic acid → 18:2(9,12)
        - Essential ω-6 fatty acid
    - α-Linolenic acid → 18:3(9,12,15)
        - Essential ω-3 fatty acid

C. Essential Fatty Acids

- Linoleic acid:
    - Precursor of ω-6 arachidonic acid
    - Arachidonic acid is the substrate for prostaglandin synthesis
- α-Linolenic acid:
    - Precursor of ω-3 fatty acids
    - Important for growth and development
- Both linoleic acid and α-linolenic acid are dietary essentials in humans because:
    - Humans lack the enzymes that can form carbon-carbon double bonds after the 9th carbon from the methyl (ω) end of a fatty acid

- CPlants provide humans with these essential fatty acids

- Note:
    - Arachidonic acid becomes essential if linoleic acid is deficient in the diet

- Essential fatty acid deficiency (rare):
    - Can result in dry, scaly dermatitis
    - Due to an inability to synthesize molecules that provide the water barrier in skin

III. Fatty Acid De Novo Synthesis

- Excess dietary carbohydrates and proteins (beyond the body's needs):
    - Can be converted to fatty acids

- In adults, de novo fatty acid synthesis occurs primarily in:
    - Liver
    - Lactating mammary glands
    - Adipose tissue (to a lesser extent)

- This process is:
    - Cytosolic
    - Endergonic
    - Reductive
- The process incorporates:
    - Carbons from acetyl coenzyme A (CoA) into the growing fatty acid chain
    - ATP
    - Reduced nicotinamide adenine dinucleotide phosphate (NADPH)
- Note:
    - Dietary TAG also supply fatty acids

A. Cytosolic Acetyl CoA Production

- First step in fatty acid synthesis:
    - Transfer of acetate units from mitochondrial acetyl CoA to the cytosol

- Mitochondrial acetyl CoA is produced by:
    - Oxidation of pyruvate
    - Catabolism of certain amino acids
- Problem:
    - The CoA portion of acetyl CoA cannot cross the inner mitochondrial membrane
- Solution:
    - Only the acetyl portion enters the cytosol, as part of citrate
- Citrate formation:
    - Formed by condensation of acetyl CoA with oxaloacetate (OAA)
    - Enzyme: Citrate synthase
- Note:
    - Citrate transport to cytosol occurs when mitochondrial citrate concentration is high
    - This happens when isocitrate dehydrogenase of the TCA cycle is inhibited by large amounts of ATP

    - Result: Accumulation of citrate and isocitrate

- Therefore:

    - Cytosolic citrate is a high-energy signal
    - Since fatty acid synthesis needs a large amount of ATP, increased ATP and citrate enhance this pathway

- In the cytosol:

    - Citrate is cleaved to:
        - Oxaloacetate (OAA)
        - Acetyl CoA
    - Enzyme: ATP citrate lyase

B. Acetyl CoA Carboxylation to Malonyl CoA

- Energy source for carbon-to-carbon condensations in fatty acid synthesis:

    - Supplied by the carboxylation and then decarboxylation of acyl groups in the cytosol

- Carboxylation of acetyl CoA to malonyl CoA:
    - Catalyzed by acetyl CoA carboxylase (ACC)
- ACC reaction details:
    - Transfers CO_2 from bicarbonate (HCO_3^-) in an ATP-requiring reaction
    - Coenzyme: Biotin (vitamin B7)
        - Biotin is covalently bound to a lysyl residue of the carboxylase
- Mechanism:
    - ACC carboxylates the bound biotin
    - Biotin then transfers the activated carboxyl group to acetyl CoA

1. Acetyl CoA Carboxylase Short-Term Regulation

- This carboxylation step is:
    - The rate-limiting step
    - A regulated step in fatty acid synthesis

- Inactive ACC = protomer (complex of ⩾2 polypeptides)

- Allosteric regulation:

    - Activated by citrate → causes protomers to polymerize
    - Inhibited by palmitoyl CoA (end product) → causes depolymerization

- Reversible phosphorylation:

    - Adenosine monophosphate-activated protein kinase (AMPK):
        - Phosphorylates and inactivates ACC
        - AMPK is:
            - Allosterically activated by AMP
            - Covalently activated by phosphorylation via several kinases
                - At least one of these AMPK kinases is activated by cAMP-dependent protein kinase A (PKA)

- Hormonal regulation:
    - In the presence of counterregulatory hormones (e.g., epinephrine and glucagon):
        - ACC is phosphorylated and inactive
    - In the presence of insulin:
        - ACC is dephosphorylated and active

- Note: This regulation is analogous to glycogen synthase regulation

2. Acetyl CoA Carboxylase Long-Term Regulation

- Prolonged consumption of excess-calorie diet (especially high-carbohydrate, low-fat):
    - Causes increase in ACC synthesis
    - Leads to increased fatty acid synthesis

- Low-calorie or high-fat, low-carbohydrate diet:
    - Has the opposite effect (decreases ACC synthesis)

- Regulatory pathways:
    - ACC synthesis is upregulated by:
        - Glucose → via carbohydrate response element-binding protein (ChREBP)
        - Insulin → via sterol regulatory element-binding protein-1c (SREBP-1c)
- Fatty acid synthase (FAS):
    - Is similarly regulated

Metformin and ACC Regulation

- Metformin (used for type 2 diabetes):
    - Lowers plasma TAG by:
        - Activating AMPK, which:
            - Inhibits ACC activity (via phosphorylation)
            - Decreases ACC and FAS expression (by reducing SREBP-1c)
    - Lowers blood glucose by:
        - Increasing AMPK-mediated glucose uptake by muscle

C. Eukaryotic Fatty Acid Synthase (FAS)

- The remaining series of reactions in fatty acid synthesis in eukaryotes:
    - Catalyzed by fatty acid synthase (FAS)
- FAS characteristics:
    - Multifunctional, homodimeric enzyme
    - Each FAS monomer:
        - Is a multicatalytic polypeptide
        - Contains six different enzymic domains
        - Contains an acyl carrier protein (ACP) domain with 4′-phosphopantetheine
- Function of FAS:
    - Adds two carbons from malonyl CoA to the carboxyl end of a series of acyl acceptors

4′-Phosphopantetheine (ACP Component)

- 4′-Phosphopantetheine:
    - A derivative of pantothenic acid (vitamin B5)
    - Carries acyl units on its terminal thiol (-SH) group
    - Presents the acyl units to the catalytic domains of FAS during fatty acid synthesis
    - Also a component of CoA

Sequential Steps of Fatty Acid Chain Elongation

Step 1:

- An acetyl group is transferred from acetyl CoA to the -SH group of the ACP domain

Step 2:

- This two-carbon fragment is then transferred to a temporary holding site

Step 3:

- The now-vacant ACP accepts a three-carbon malonyl group from malonyl CoA

Step 4:

- The acetyl group (on the cysteine residue) condenses with the malonyl group (on ACP)
- CO_2 is released — this CO_2 was originally added by acetyl CoA carboxylase (ACC)
- The result is a four-carbon unit attached to the ACP domain

Note: The next three reactions (steps 5–7) convert the 3-ketoacyl group into a saturated acyl group

Involve two NADPH-requiring reductions and one dehydration step

Step 5

- The keto group is reduced to an alcohol

Step 6

- A molecule of water is removed

- This creates a trans double bond between:
    - Carbon 2 (α-carbon)
    - Carbon 3 (β-carbon)

Step 7

- The double bond is reduced
- Produces a four-carbon group (butyryl):
    - Three terminal carbons are fully saturated
    - Group remains attached to the ACP domain

Cycle Repeats (Marked by Asterisk *)

- Repetition begins with:
    - Transfer of butyryl unit from ACP to cysteine residue [2*]
    - Attachment of a malonyl group to ACP [3*]
    - Condensation of the two groups → CO_2 is released [4*]

- The newly formed carbon chain then undergoes:
    - Reduction of β-carbonyl [5*]
    - Dehydration [6*]
    - Reduction [7*]
- This sequence generates hexanoyl-ACP

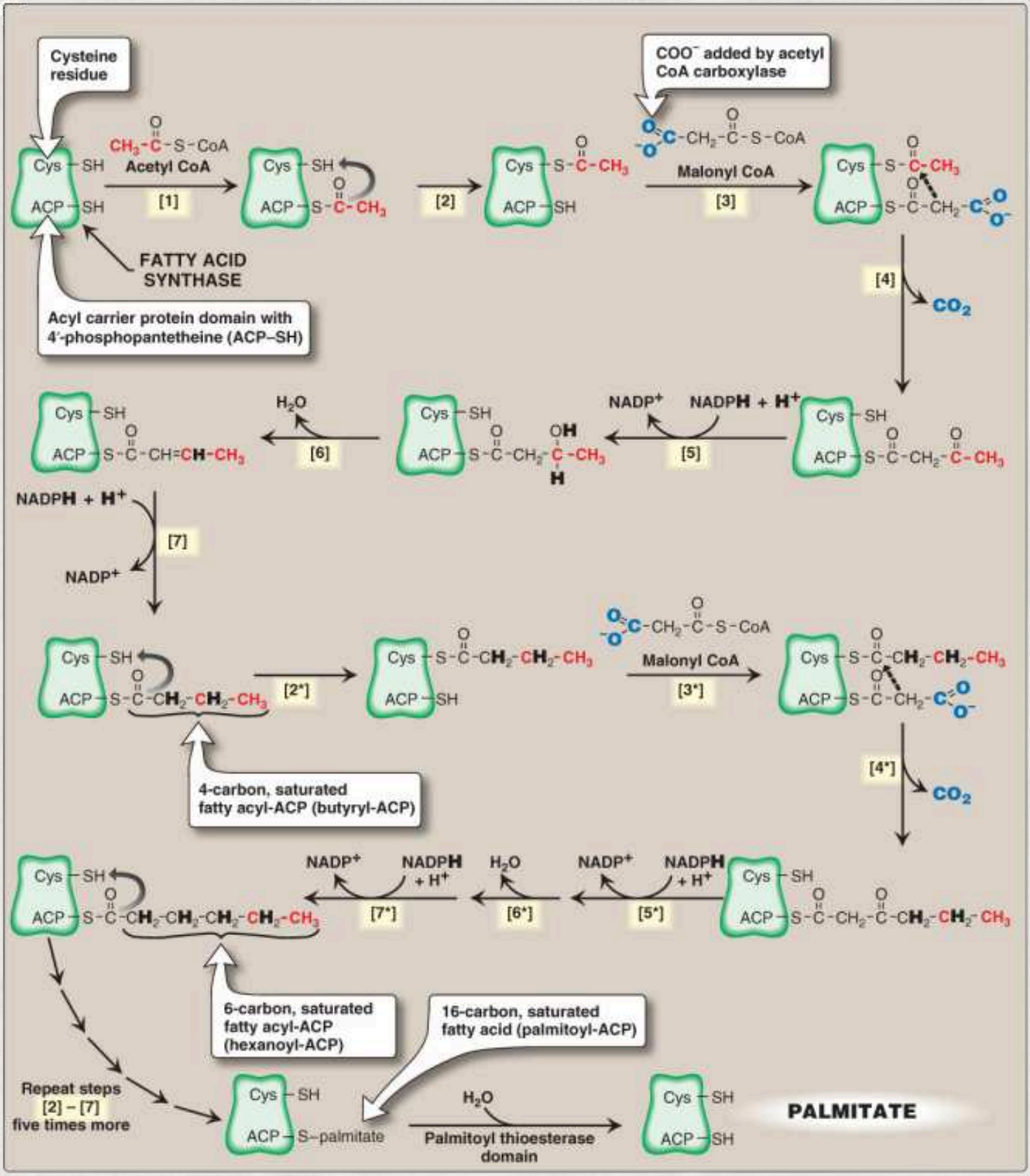
Elongation Process

- The cycle continues, repeating steps [2*] through [7*]
- Continues until the fatty acid chain reaches 16 carbons in length

Final Step of FAS

- The final catalytic activity of FAS:
    - Cleaves the thioester bond
    - Releases a fully saturated molecule of palmitate (16:0)

Synthesis of Palmitate (16:0) by Multifunctional Fatty Acid Synthase



Important Note:

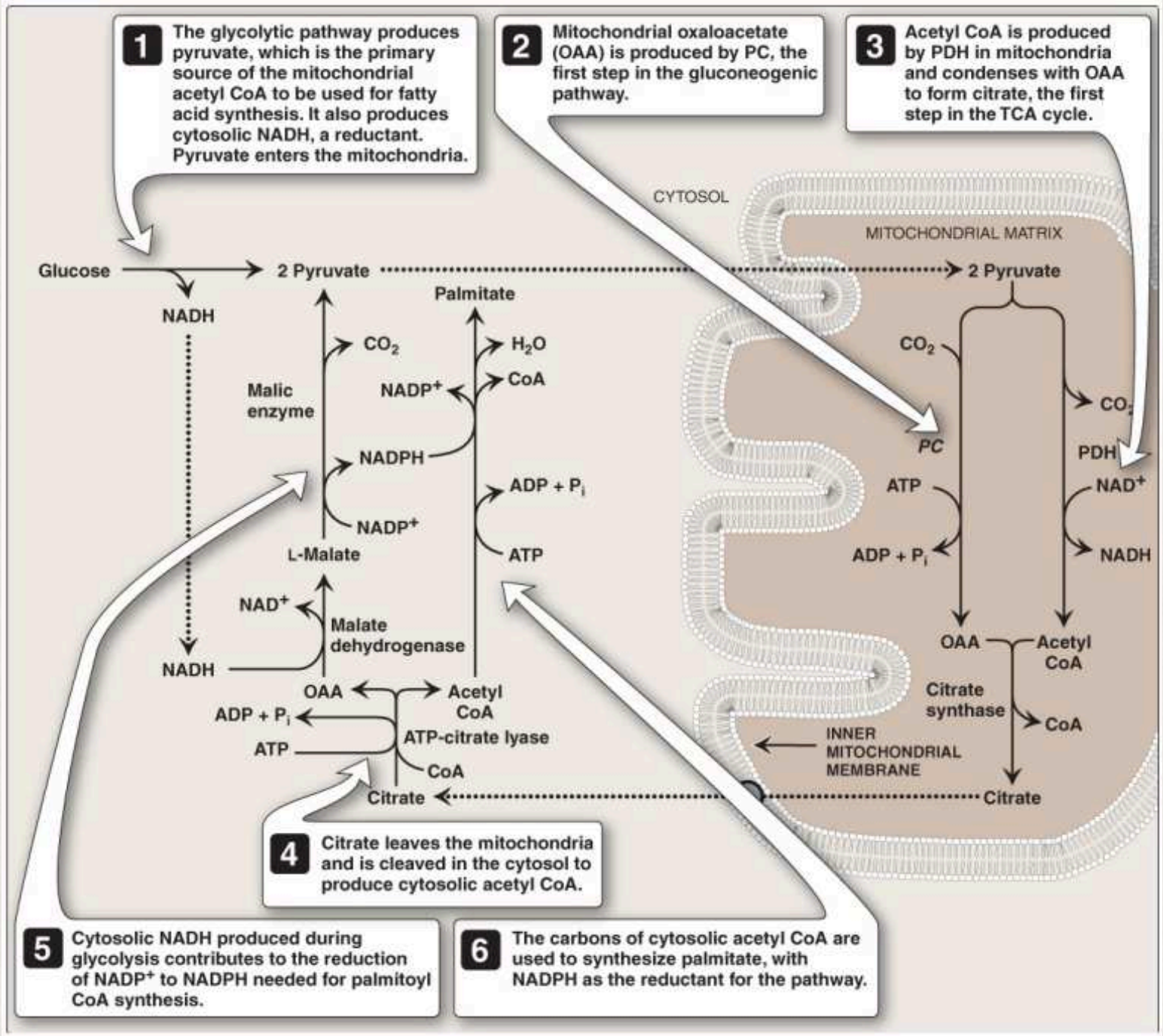
- All carbons in palmitic acid come from malonyl CoA
    - Except the two carbons donated by the original acetyl CoA (the first acyl acceptor)
    - These two are located at the methyl (ω) end of the fatty acid
- This highlights the rate-limiting nature of the ACC reaction
- Shorter-length fatty acids:
    - Are produced only in the lactating mammary gland

D. Reductant Sources

- Synthesis of one palmitate requires:
    - 14 NADPH (reductant/reducing agent)
- Major source of NADPH:
    - Pentose phosphate pathway
        - Two NADPH are produced per molecule of glucose 6-phosphate entering the pathway

- Additional cytosolic NADPH production:
    - From the conversion of malate to pyruvate
        - Malate is oxidized and decarboxylated by:
            - Cytosolic malic enzyme
                - ($NADP^+$-dependent malate dehydrogenase)
        - Produces:
            - Cytosolic NADPH
            - CO_2
- Note:
    - Malate can arise from reduction of oxaloacetate (OAA) by:
        - Cytosolic NADH-dependent malate dehydrogenase
- Source of cytosolic NADH:
    - Glycolysis
- Source of OAA:
    - From citrate cleavage by ATP citrate lyase

Interrelationship between Glucose Metabolism and Palmitate Synthesis



E. Further Elongation

- Palmitate (16:0):
    - A 16-carbon, fully saturated long-chain fatty acid (LCFA)
    - It is the primary end product of FAS activity
- Further elongation of palmitate:
    - Occurs by addition of two-carbon units to the carboxylate end
    - Takes place primarily in the smooth endoplasmic reticulum (SER)
- Elongation process:
    - Requires a system of separate enzymes
        - Not a multifunctional enzyme like FAS
    - Malonyl CoA serves as the two-carbon donor
    - NADPH supplies the electrons

- Brain-specific elongation:
    - The brain has additional elongation capabilities
    - Can produce very long-chain fatty acids (VLCFA):
        - VLCFA = fatty acids over 22 carbons
        - Required for synthesis of brain lipids

F. Chain Desaturation

- Enzymes involved:
    - Fatty acyl CoA desaturases, located in the smooth endoplasmic reticulum (SER)
- Function:
    - Desaturate long-chain fatty acids (LCFA) by adding cis double bonds
- Desaturation reaction requirements:
    - Oxygen (O_2)
    - NADH
    - Cytochrome b_5
    - FAD-linked reductase (flavin adenine dinucleotide-linked)

- Reaction mechanism:
    - Both the fatty acid and NADH are oxidized
    - O_2 is reduced to H_2O
- Typical desaturation site:
    - Between carbons 9 and 10
    - Produces:
        - Oleic acid, 18:1(9)
        - Palmitoleic acid, 16:1(9) (in smaller amounts)
- Polyunsaturated fatty acid (PUFA) synthesis:
    - Achieved through additional desaturation combined with elongation
- Human desaturase enzymes present:
    - Carbon 9, 6, 5, and 4 desaturases
- Limitation:
    - Humans lack the ability to insert double bonds between carbon 10 and the ω end of the chain

- Nutritional consequence:
    - Explains the essentiality of:
        - ω-6 linoleic acid
        - ω-3 linolenic acid

G. Storage as TAG Components

- Mono-, di-, and triacylglycerols:
    - Comprise one, two, or three fatty acids esterified to a molecule of glycerol

- Esterification:
    - Fatty acids are esterified through their carboxyl groups
    - Results in:
        - Loss of negative charge
        - Formation of neutral fat

- Note:
    - An acylglycerol that is:
        - Solid at room temperature = fat
        - Liquid at room temperature = oil

1. Arrangement of Fatty Acids in TAG

- Triacylglycerol (TAG) = Three fatty acids esterified to a glycerol molecule
- Fatty acids are usually not of the same type:
  - Carbon 1 → typically saturated
  - Carbon 2 → typically unsaturated
  - Carbon 3 → can be either saturated or unsaturated
- Note:
  - Presence of unsaturated fatty acid(s) lowers the Tm (melting temperature) of the lipid

2. Triacylglycerol Storage and Function

- Solubility:
  - TAGs are only slightly soluble in water
  - Cannot form stable micelles on their own

- Storage in white adipocytes:
    - TAGs coalesce to form large oily, nearly anhydrous droplets
    - Stored in cytosolic lipid droplets
    - These droplets are the major energy reserve of the body

- Note:
    - TAGs in brown adipocytes are used for heat production through nonshivering thermogenesis

3. Glycerol 3-Phosphate Synthesis

- Glycerol 3-phosphate:
    - Is the initial acceptor of fatty acids during TAG synthesis

- Two major pathways for glycerol 3-phosphate production:

A. From Glucose (in Liver and Adipose Tissue)

- Glucose → DHAP via glycolytic pathway

- DHAP is reduced by glycerol 3-phosphate dehydrogenase → Glycerol 3-phosphate

B. From Free Glycerol (in Liver only)

- Free glycerol is converted to glycerol 3-phosphate
    - Enzyme: Glycerol kinase
- Note:
    - This pathway is absent in adipose tissue

Additional Notes

- Glyceroneogenesis:
    - A third process for glycerol 3-phosphate synthesis
- Insulin Dependence in Adipocytes:
    - GLUT-4 (glucose transporter in adipocytes) is insulin dependent

- When plasma glucose is low, adipocytes:
  - Have limited ability to synthesize glycerol phosphate
  - Therefore, cannot produce TAG de novo

4. Fatty Acid Activation

- A free fatty acid (FFA) must be converted to its activated form before participating in metabolic processes such as TAG synthesis

- Activated form:

  - Fatty acid bound to Coenzyme A (CoA) via a thioester bond

- Catalyzing enzyme:

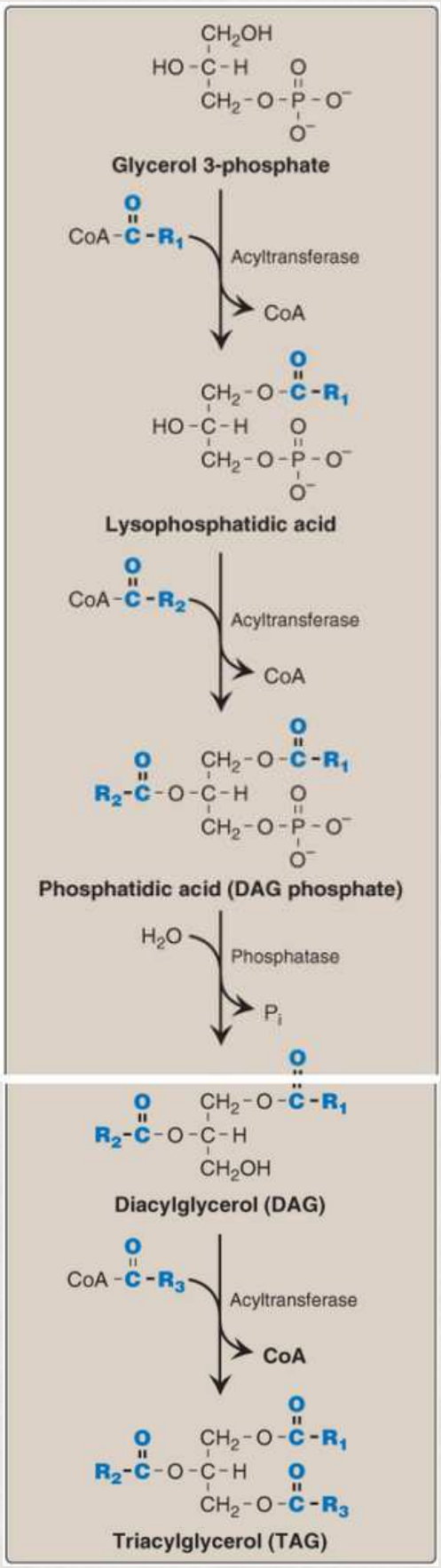
  - A family of fatty acyl CoA synthetases (also called thiokinases)

5. Triacylglycerol (TAG) Synthesis

- Pathway begins from glycerol 3-phosphate

- Involves four reactions:
    - Addition of 1st fatty acid from fatty acyl CoA
    - Addition of 2nd fatty acid from fatty acyl CoA
    - Removal of phosphate group
    - Addition of third fatty acid

Synthesis of TAG



H. Triacylglycerol Fate in Liver and Adipose Tissue

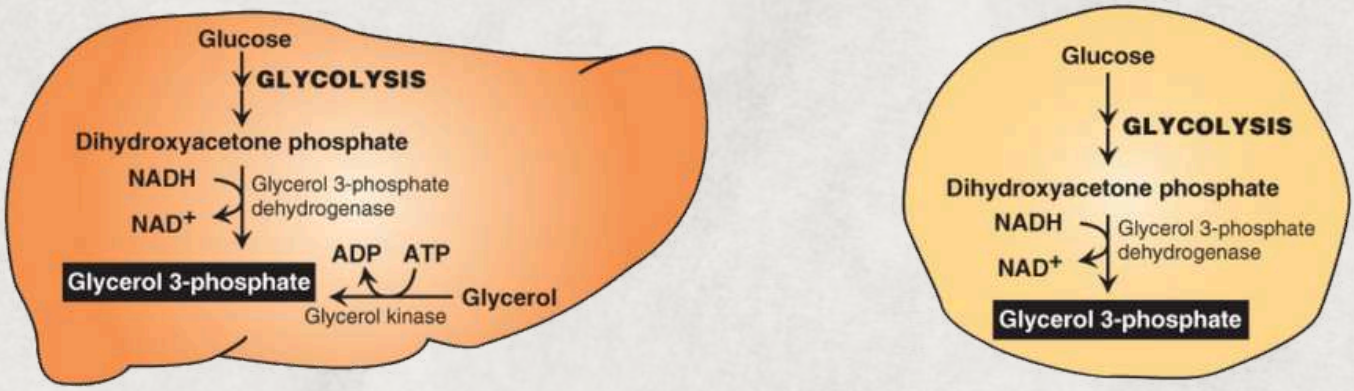
In White Adipose Tissue (WAT)

- TAG storage:
    - Stored as nearly anhydrous fat droplets in the cytosol
- Fat droplet structure:
    - Coated with perilipins:
        - A family of proteins that sequester and protect TAG from lipolysis
        - Activated only when the body requires fatty acids for fuel
- Clinical relevance:
    - Perilipins may play a role in:
        - Type 2 diabetes
        - Atherosclerosis
        - Cardiovascular disease

In Liver

- Healthy liver:
    - Stores little TAG
- Most TAG in liver is:
    - Exported, packaged with other lipids and apolipoproteins
    - Forms very-low-density lipoproteins (VLDL)
- VLDL characteristics:
    - Nascent VLDL are secreted directly into the blood
    - Mature in blood and deliver endogenously derived lipids to peripheral tissues
- Note:
    - Chylomicrons carry dietary (exogenous) lipids

Pathways for Production of Glycerol 3-phosphate in Liver and Adipose Tissue



IV. Fat Mobilization And Fatty Acid Oxidation

- Stored fatty acids in white adipose tissue (WAT):
    - Stored as neutral triacylglycerol (TAG)
    - Serve as the body's major fuel storage reserve
- Why TAGs are efficient energy stores:
    - Highly reduced
    - Largely anhydrous

- Energy yield from complete oxidation:
    - Fatty acids → CO_2 + H_2O = 9 kcal/g fat
    - Compared to:
        - 4 kcal/g for protein or carbohydrate

A. Fatty Acid Release from Fat

- Lipolysis:
    - Required to release free fatty acids (FFA) and glycerol from TAG
    - Carried out by perilipins and lipases

- Steps of lipolysis:

a. Initiated by adipose triglyceride lipase (ATGL):

- Produces diacylglycerol (DAG)
- DAG is the preferred substrate for:

a. Hormone-sensitive lipase (HSL):

- Produces monoacylglycerol (MAG)

a. MAG lipase acts on MAG to complete hydrolysis

1. Regulation of Perilipins and HSL

- PKA-mediated phosphorylation:
    - Both perilipins and HSL are phosphorylated by PKA
    - PKA is activated by cAMP, which is produced when:
        - Catecholamines (e.g., epinephrine) bind to β-adrenergic receptors
        - Activate adenylyl cyclase

- Effects of phosphorylation:
    - Phosphorylated perilipin allows translocation and binding of phosphorylated (active) HSL to lipid droplet
    - Process similar to glycogen phosphorylase activation

- cAMP cascade effects:
    - Inhibits ACC (acetyl CoA carboxylase)
    - Turns off fatty acid synthesis
    - Turns on TAG degradation

- Insulin effects:
    - High plasma insulin:
        - Causes dephosphorylation and inactivation of HSL
        - Suppresses ATGL expression

2. Fate of Glycerol

- Adipocytes lack glycerol kinase:
    - Cannot metabolize glycerol
- Glycerol is transported to the liver:
    - Liver contains glycerol kinase
    - Converts glycerol → glycerol 3-phosphate
- Fate of glycerol 3-phosphate in liver:
    - Used to form TAG
    - Or converted to DHAP by glycerol 3-phosphate dehydrogenase (reverse reaction)
    - DHAP can enter:
        - Glycolysis
        - Gluconeogenesis

3. Fate of Fatty Acids

- Free fatty acids (FFA):
    - Exit adipocyte → enter blood → bind serum albumin
- FFA transported to:
    - Tissues like muscle
    - Inside cells:
        - Activated to CoA derivatives
        - Oxidized for energy in mitochondria
- Tissues that do not utilize plasma FFA:
    - Red blood cells (RBCs):
        - No mitochondria
    - Brain:
        - Uses little/no fatty acids
        - Reason not well understood
- Reesterification of fatty acids:
    - >50% of fatty acids released from adipose TAG are reesterified to glycerol 3-phosphate

- Problem: WAT does not express glycerol kinase

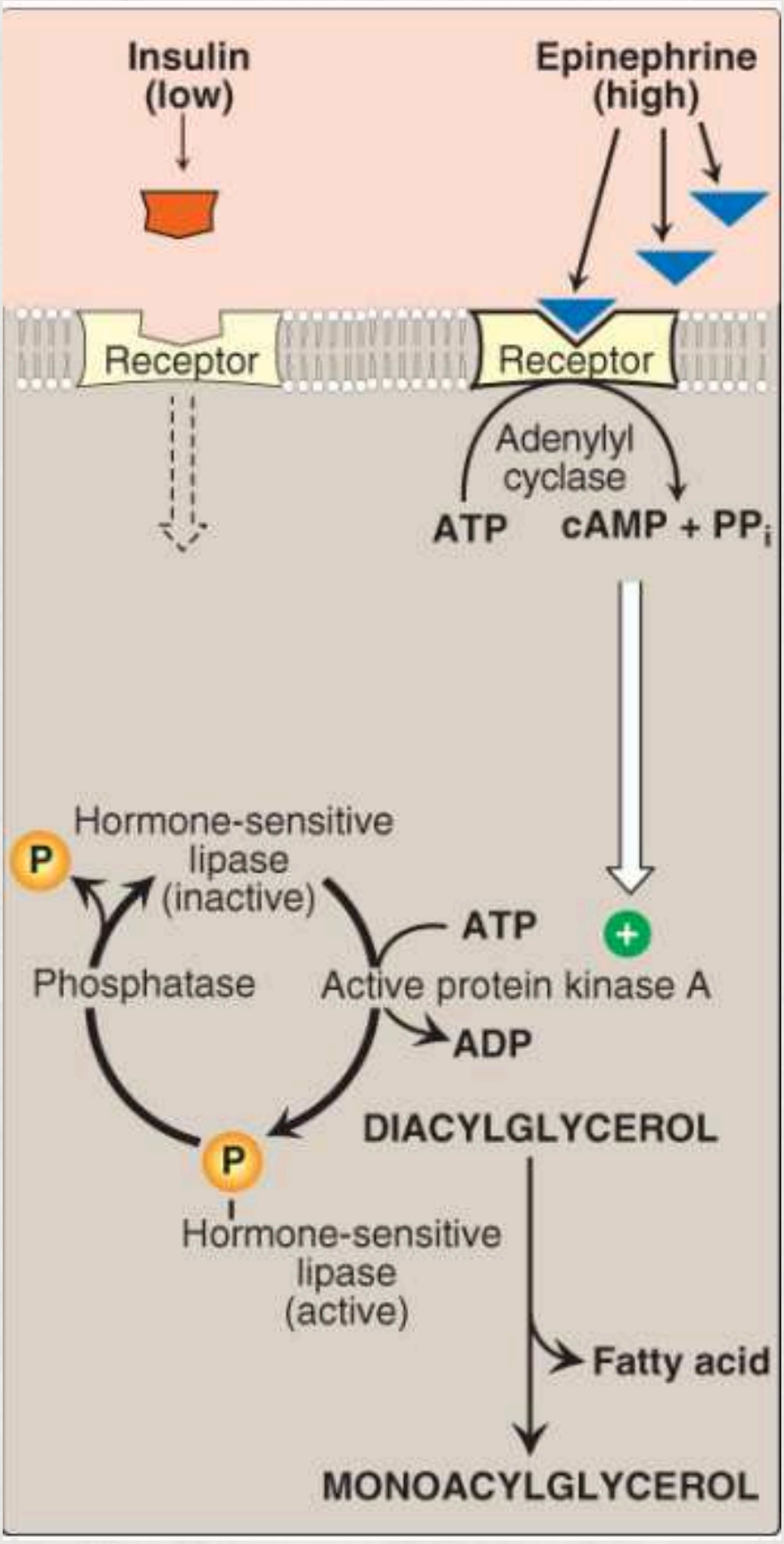
- Solution: Glycerol 3-phosphate is produced by glyceroneogenesis aka incomplete gluconeogenesis:

    - Incomplete gluconeogenesis:
        - Pyruvate → oxaloacetate (OAA) via pyruvate carboxylase
        - OAA → phosphoenolpyruvate (PEP) via PEP carboxykinase
        - PEP → DHAP (via shared glycolytic/gluconeogenic steps)
        - DHAP → glycerol 3-phosphate

- Clinical relevance:

    - Reesterification reduces plasma FFA levels
    - Elevated FFA levels are associated with:
        - Insulin resistance
        - Type 2 diabetes
        - Obesity

Hormonal Regulation of Diacylglycerol Degradation in the Adipocyte



B. Fatty Acid β-Oxidation

- Definition:
    - The major pathway for catabolism of fatty acids
    - Occurs in the mitochondria
    - Process: Two-carbon fragments are successively removed from the carboxyl end of fatty acyl CoA
- Products:
    - Acetyl CoA
    - NADH
    - $FADH_2$

I. Long-Chain Fatty Acid Transport into Cytosol and Mitochondria

A. Cellular Uptake of Fatty Acids

- Mechanisms:
    - Passive diffusion
    - Lipid transport proteins:
        - Fatty acid translocase (FAT)
        - Fatty acid-binding protein (FABP)
        - Fatty acid transport protein (FATP)

- Long-chain fatty acids (LCFA):
    - Taken up specifically by FATP

B. Activation of Fatty Acids

- Once LCFA enter the cell:
    - Converted in the cytosol to fatty acyl CoA
- Enzyme: Long-chain fatty acyl CoA synthetase (thiokinase)
    - Located on the outer mitochondrial membrane

C. Mitochondrial Transport Requirement

- β-Oxidation occurs in the mitochondrial matrix
- Inner mitochondrial membrane is impermeable to CoA
- Therefore, the acyl group must be transported via a specialized carrier

D. Carnitine Shuttle (Rate-limiting Transport Process)

- Carrier: Carnitine
- Name of process: Carnitine shuttle

a. Translocation Steps (Three Key Steps)

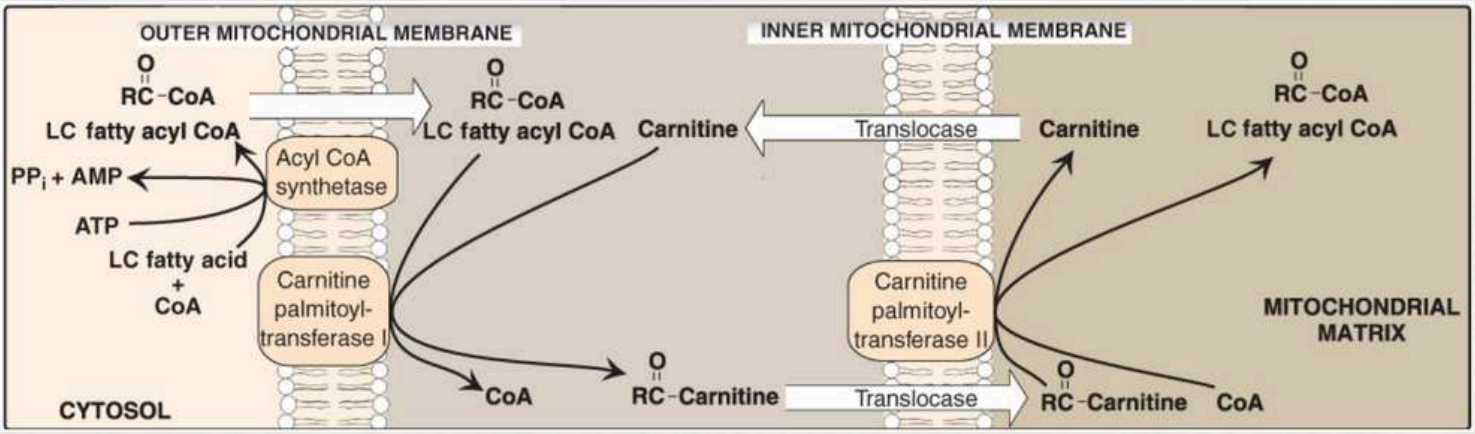
1. Transfer of acyl group to carnitine:
    - Enzyme: Carnitine palmitoyltransferase I (CPT-I)
        - Also known as carnitine acyltransferase I (CAT-I)
        - Located on the outer mitochondrial membrane
    - Reaction:
        - Acyl group from CoA → Carnitine
        - Forms acylcarnitine
        - Regenerates free CoA

2. Transport across inner mitochondrial membrane:
    - Transporter: Carnitine-acylcarnitine translocase
    - Exchanges acylcarnitine into matrix and free carnitine out

3. Transfer of acyl group back to CoA:

- Enzyme: Carnitine palmitoyltransferase II (CPT-II)
  - Also known as carnitine acyltransferase II (CAT-II)
  - Located on the inner mitochondrial membrane
- Reaction:
  - Acyl group from carnitine → CoA in matrix
  - Regenerates free carnitine

Carnitine Shuttle



b. Carnitine Shuttle Inhibitor

- Inhibitor: Malonyl CoA
  - Inhibits CPT-I (Carnitine Palmitoyltransferase I)
  - Prevents entry of long-chain acyl groups into the mitochondrial matrix

- Functional Significance:
    - When fatty acid synthesis is occurring in the cytosol (indicated by presence of malonyl CoA):
        - The newly made palmitate cannot be transferred into mitochondria
        - Prevents degradation of newly synthesized fatty acids
- Tissue-Specific Notes:
    - Muscle tissue:
        - Does not synthesize fatty acids
        - But contains mitochondrial isozyme of ACC (ACC2)
        - Allows regulation of β-oxidation
    - Liver:
        - Contains both isozymes of ACC
- Additional Regulation:
    - Regulated by the acetyl CoA / CoA ratio:
        - As the ratio increases, the CoA-requiring thiolase reaction decreases

c. Carnitine Sources

- Dietary source:
    - Carnitine is found primarily in meat products
- Endogenous synthesis:
    - Synthesized from lysine and methionine
    - Enzymatic pathway is present in:
        - Liver
        - Kidneys
    - Not synthesized in:
        - Skeletal muscle
        - Cardiac muscle
- Tissue dependency:
    - Skeletal and cardiac muscles are:
        - Totally dependent on carnitine uptake from:
            - Endogenous synthesis (via blood)
            - Diet
- Distribution note:
    - Skeletal muscle contains 97% of all carnitine in the body

Carnitine Transport into Cells

- Transport mechanism: Carnitine enters cells via carnitine transporters
- High-affinity transporter (in heart, muscle, kidney):
    - Organic cation transporter novel 2 (OCTN2)
- Liver transporter:
    - Different, low-affinity, high-capacity carnitine transporter
- Primary carnitine deficiency:
    - Due to defect in OCTN2
    - Leads to:
        - Urinary loss of carnitine
        - Low serum and cellular carnitine levels

d. Carnitine Deficiencies

1. Primary Carnitine Deficiency

- Cause: Defects in membrane transporter

    - Prevents carnitine uptake by:
        - Cardiac muscle
        - Skeletal muscle
        - Kidneys

- Effect: Carnitine is excreted in urine

- Treatment: Carnitine supplementation

2. Secondary Carnitine Deficiency

- Primary cause: Defects in fatty acid oxidation

    - Leads to accumulation of acylcarnitines
    - Acylcarnitines are excreted in urine
    - Result: Decreased carnitine availability

- Acquired causes:

    - Liver disease → Decreased carnitine synthesis
    - Valproic acid (antiseizure drug) → Decreased renal reabsorption

3. CPT-I and CPT-II Deficiencies (Defects in mitochondrial oxidation)

- CPT-I deficiency:
    - Affects the liver
    - Impaired LCFA usage for fuel
    - Severe effect on hepatic glucose synthesis during fasting (an endergonic process)
    - Leads to:
        - Severe hypoglycemia
        - Coma
        - Death
- CPT-II deficiency:
    - Affects:
        - Liver
        - Cardiac muscle
        - Skeletal muscle
    - Most common form: Affects skeletal muscle (least severe)
        - Presents as:
            - Muscle weakness
            - Myoglobinemia after prolonged exercise

- Treatment:
    - Avoid fasting
    - High-carbohydrate, low-fat diet
    - Supplement with medium-chain TAG

2. Entry of Shorter-Chain Fatty Acids into Mitochondria

- Fatty acids ⩽12 carbons:
    - Can cross inner mitochondrial membrane without carnitine or CPT system
- Once inside mitochondria:
    - Activated to CoA derivatives by matrix enzymes
    - Undergo oxidation
- Note:
    - Medium-chain fatty acids:
        - Plentiful in human milk
        - Oxidation is independent of CPT-I
        - Therefore, malonyl CoA does not inhibit their oxidation

3. β-Oxidation Reactions

- Overview:
    - Involves four sequential reactions at the β-carbon (carbon 3)
    - Result: Shortens the fatty acid by two carbons at the carboxylate end

Steps of β-Oxidation:

1. Oxidation:
    - Produces $FADH_2$

2. Hydration

3. Second oxidation:
    - Produces NADH

4. CoA-dependent thiolytic cleavage:
    - Releases one molecule of acetyl CoA

- Enzyme Specificity:
    - Each step is catalyzed by enzymes with chain-length specificity
- Note:
    - For long-chain fatty acids (LCFA), the last three steps are catalyzed by a trifunctional protein

Cycle Repetition:

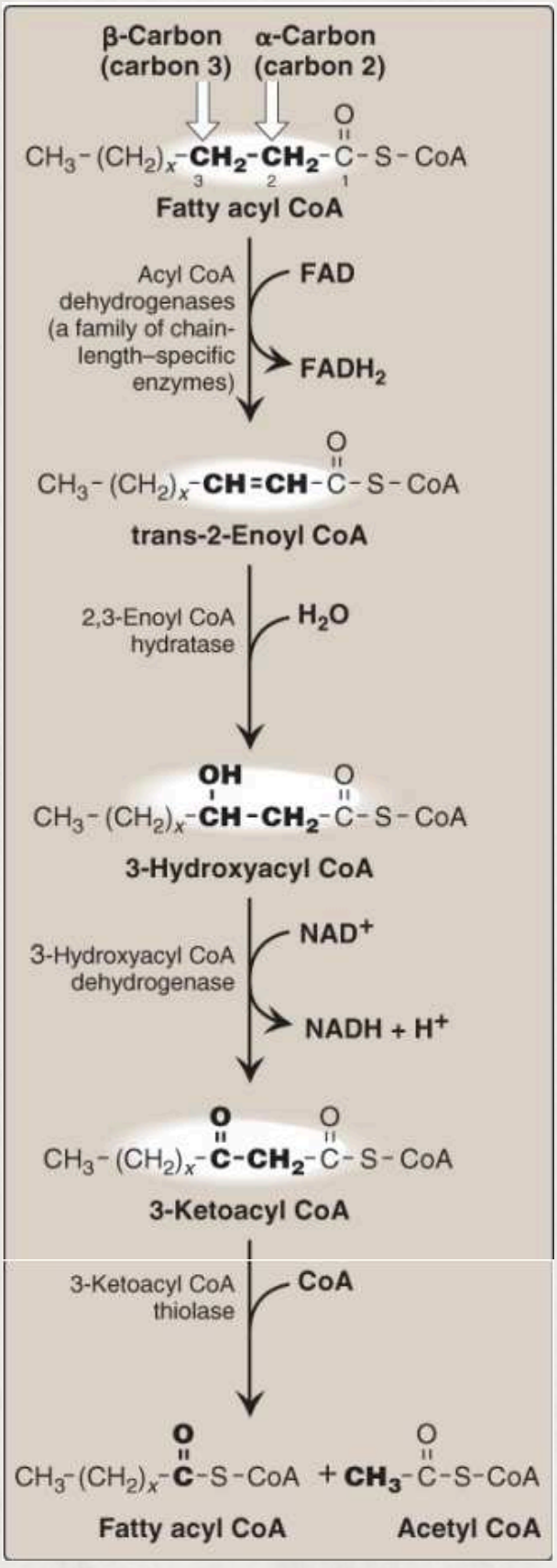
- For saturated fatty acids with even-numbered carbon chains:
    - Number of cycles = (n/2) - 1 (where n = number of carbons)
- Each cycle yields:
    - 1 acetyl CoA
    - 1 NADH
    - 1 $FADH_2$

- Final cycle produces:
    - 2 acetyl CoA

Fates of Acetyl CoA and Reduced Coenzymes:

- Acetyl CoA can be:
    - Oxidized
    - Used in hepatic ketogenesis
- Reduced coenzymes:
    - NADH oxidized by ETC Complex I
    - $FADH_2$ oxidized by coenzyme Q
- Note:
    - Acetyl CoA is a positive allosteric effector of pyruvate carboxylase (see Chapter 10)
    - This links fatty acid oxidation with gluconeogenesis

Enzymes involved in the β-oxidation of fatty acyl coenzyme A (CoA)



4. β-Oxidation Energy Yield

- Fatty Acid β-Oxidation Produces High Energy:
  - The energy yield from fatty acid β-oxidation is very high.

Example: Complete Oxidation of Palmitoyl CoA ($C_{16:0}$)

- Yields the following:
  - 8 acetyl CoA
  - 7 NADH
  - 7 $FADH_2$
- These products can generate a total of 131 ATP.
- Activation of the fatty acid consumes 2 ATP, so:
  - Net ATP yield = 129 ATP.

Energy Calculation for even-numbered aturated fatty acid

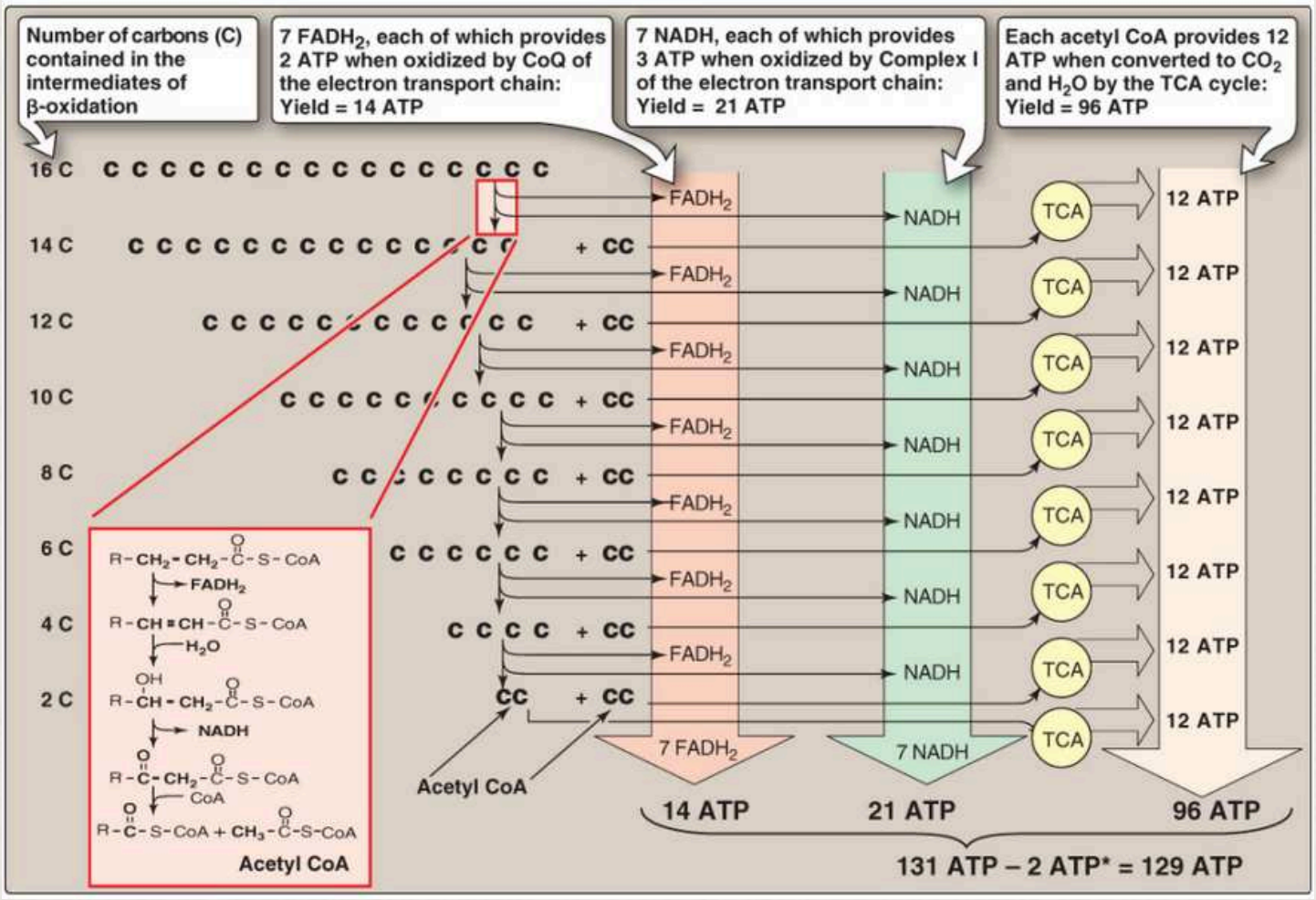
- Let n = number of carbon atoms in the saturated fatty acid (even-numbered).

- Use the following steps:
    - Number of β-oxidation cycles = (n / 2) – 1
        - Each cycle removes 2 carbons as 1 acetyl CoA.
        - So for C_{16}, (16 / 2) – 1 = 7 cycles.
    - Acetyl CoA produced = (n / 2)
        - One additional acetyl CoA is produced in the final cycle, when a 4-carbon fatty acyl CoA is cleaved into two acetyl CoA.
        - So for palmitate (C_{16}), you get 8 acetyl CoA.
    - NADH and $FADH_2$ = number of β-oxidation cycles
        - Each of the 7 cycles produces:
            - 1 NADH = 7 NADH (total)
            - 1 $FADH_2$ = 7 $FADH_2$ (total)

ATP Yield Summary for Palmitate (C_{16})

Molecule	Quantity	ATP per molecule	Total ATP
Acetyl CoA	8	12	96
NADH	7	3	21
$FADH_2$	7	2	14
Subtotal	—	—	131 ATP
Activation cost	—	—	-2 ATP
Grand total	—	—	Net: 129 ATP

Summary of the Energy Yield from the Oxidation of Palmitoyl Coenzyme A (CoA) (16 carbons)



Note: Activation of palmitate to palmitoyl CoA requires the equivalent of 2 ATP [ATP → AMP + PPi

5. Medium-Chain Fatty Acyl CoA Dehydrogenase (MCAD) Deficiency

- Fatty Acyl CoA Dehydrogenases in Mitochondria:
    - There are four species of fatty acyl CoA dehydrogenases.
    - Each has distinct but overlapping specificity for:
        - Short-chain fatty acids
        - Medium-chain fatty acids
        - Long-chain fatty acids
        - Very-long-chain fatty acids

MCAD Deficiency

- Most common inborn error of β-oxidation
- Genetic basis:
    - Autosomal recessive disorder
- Prevalence:
    - Found in 1:14,000 births worldwide
    - Higher incidence in Caucasians of Northern European descent

Pathophysiology

- Impaired oxidation of fatty acids with 6 to 10 carbons
- Results in:
    - Decreased production of acetyl CoA
    - Increased reliance on glucose for energy
        - Leads to hypoketotic hypoglycemia

Laboratory Findings

- Urine studies show:
    - Accumulation of medium-chain acyl carnitines
    - Presence of medium-chain dicarboxylic acids

Treatment:

- Avoidance of fasting

6. Oxidation of Fatty Acids with an Odd Number of Carbons

- Follows same β-oxidation steps as even-numbered fatty acids.

- Continues until final three-carbon fragment: Propionyl CoA.
    - Also produced during the metabolism of some amino acids

Three-Step Metabolism of Propionyl CoA:

a. D-Methylmalonyl CoA Synthesis

- Enzyme: Propionyl CoA carboxylase
- Reaction:

  Propionyl CoA + CO_2 + ATP → D-Methylmalonyl CoA

- Cofactors:
    - Biotin (Vitamin B7) — same as in acetyl CoA carboxylase (ACC)
    - ATP

b. L-Methylmalonyl CoA Formation

- Enzyme: Methylmalonyl CoA racemase
- Converts D-isomer to L-isomer

c. Succinyl CoA Synthesis

- Enzyme: Methylmalonyl CoA mutase
- Reaction:

L-Methylmalonyl CoA → Succinyl CoA

- Cofactor: Vitamin B12 (deoxyadenosylcobalamin)
    - One of only two B12-dependent enzymes in humans

Clinical Correlation: Gluconeogenesis

- Succinyl CoA enters the TCA cycle
- → Only known glucogenic product of fatty acid oxidation

Vitamin B12-Related Notes

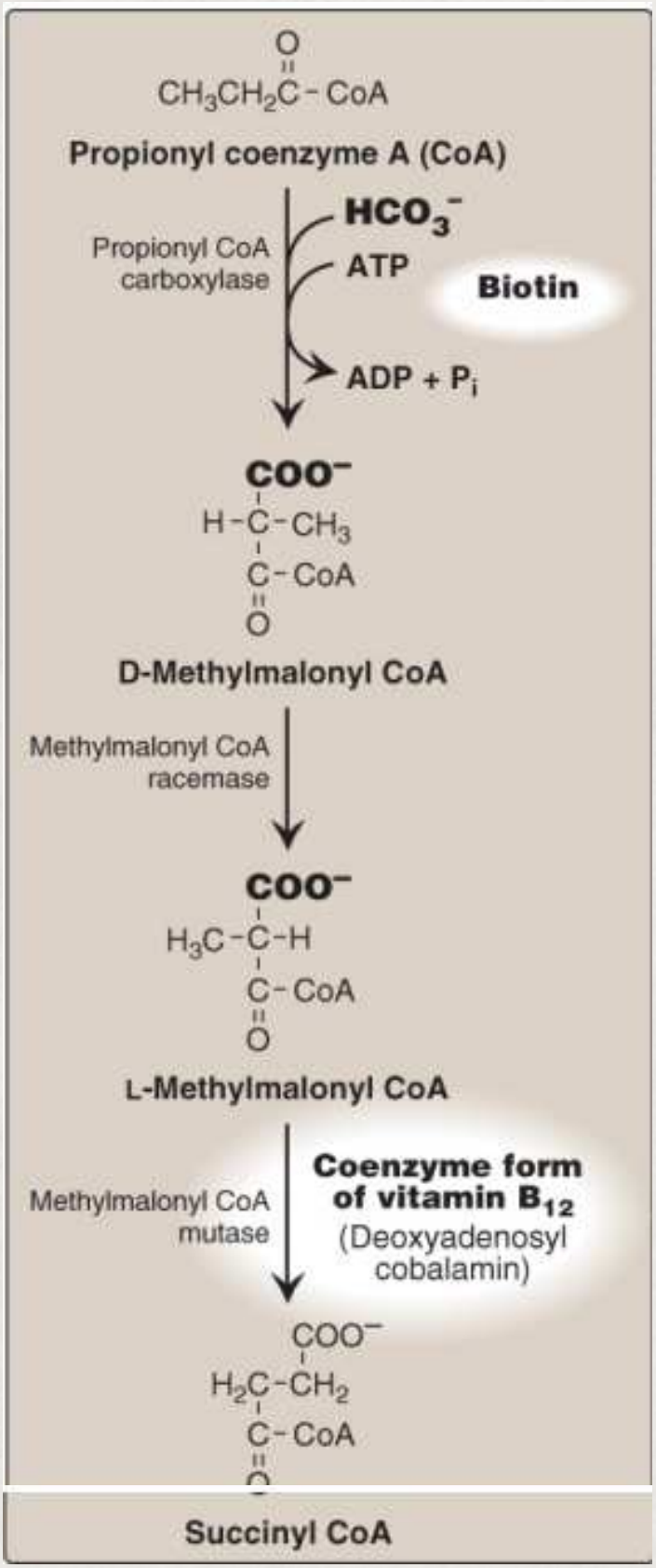
- Second B12-dependent enzyme: Methionine synthase
    - Converts homocysteine → methionine
    - Essential for:
        - Folate recycling
        - Conversion of B12 to coenzyme form

- Deficiency consequences:
    - Early: Hematologic abnormalities (similar to folate deficiency)
    - Late: Neurologic symptoms: paresthesias, numbness, ataxia
- Diagnostic markers:
    - ↑ Methylmalonic acid (MMA) in serum = B12 deficiency
    - Both propionic acid and MMA appear in urine
    - MMA helps differentiate B12 deficiency from folate deficiency

Inherited Disorders

- Methylmalonic acidemia/aciduria:
    - Caused by:
        - Defects in methylmalonyl CoA mutase
        - Deficiency of methionine synthase

Metabolism of propionyl CoA



7. β-Oxidation of Unsaturated Fatty Acids

- Challenge: Unsaturated FA oxidation forms intermediates that cannot be acted on by 2,3-enoyl CoA hydratase (step 2 of β-oxidation).

a. Odd-Numbered Double Bonds

- Example: Oleic acid = 18:1(9)
- After 3 β-oxidation rounds → Intermediate: 3-cis-enoyl CoA
- Solution:
    - Enzyme: 3,2-enoyl CoA isomerase
    - Converts 3-cis → 2-trans, the correct substrate for hydratase

b. Even-Numbered Double Bonds

- Example: Linoleic acid = 18:2(9,12)
- Problem: Produces 2,4-dienoyl CoA intermediate, which is not a substrate

- Solution:
    - Enzymes required:
        - 3,2-enoyl CoA isomerase
        - 2,4-dienoyl CoA reductase (requires NADPH)

- Note: Unsaturated FAs are less reduced → fewer NADH/$FADH_2$ → less ATP yield

8. Peroxisomal β-Oxidation (for VLCFA ⩾22 carbons)

- VLCFAs are first oxidized in peroxisomes, not mitochondria.

a. Activation

- Peroxisomes contain acyl CoA synthetases for VLCFA.

- VLCFA-CoA is formed and enters peroxisomal β-oxidation.

b. Comparison: Mitochondrial vs. Peroxisomal β-Oxidation

Feature	Mitochondrial β-Oxidation	Peroxisomal β-Oxidation
Primary Substrate	Short-, medium-, and long-chain fatty acids ($\leqslant$ 22 C)	Very-long-chain fatty acids (VLCFA $\geqslant$ 22 C)
Site of Activation	Cytosol/outer mitochondrial membrane	Peroxisome
Initial Dehydrogenation Enzyme	Acyl CoA dehydrogenase (produces $FADH_2$)	Acyl CoA oxidase (produces $FADH_2$ used to reduce $O_2 \rightarrow H_2O_2$)
Electron Acceptor	ETF $\rightarrow$ ETC $\rightarrow$ ATP	O_2 (no ATP generated directly)
Hydrogen Peroxide Formation	Not formed	H_2O_2 formed; detoxified by catalase to H_2O
End Products	Acetyl CoA, NADH, $FADH_2$	Shortened fatty acyl-CoA (e.g., octanoyl-CoA) + H_2O_2
Further Oxidation Site	Complete within mitochondria	Requires transfer to mitochondria (via carnitine) for completion
Clinical Relevance	Impaired in mitochondrial disorders	Defective in Zellweger syndrome, X-linked ALD

(ETF = Electron Transfer Flavoprotein)

End Product: Shortened FA (linked to carnitine) → sent to mitochondria for completion of oxidation

c. Clinical Correlations

- Zellweger Syndrome:
    - Cause: Peroxisomal biogenesis defect
    - Result: Impaired import of matrix proteins
    - Consequence: ↑ VLCFA in blood and tissues
- X-linked Adrenoleukodystrophy (ALD):
    - Cause: Defective VLCFA transport into peroxisomes
    - Consequence: Accumulation of VLCFA
    - Affects: Primarily brain and adrenal cortex

C. Peroxisomal α-Oxidation

- Substrate: Phytanic acid
    - A branched-chain fatty acid derived from chlorophyll metabolism
    - Problem: Methyl group on β-carbon prevents β-oxidation

1. α-Oxidation Mechanism

- Step 1: Hydroxylation at α-carbon (carbon-2)
    - Enzyme: Phytanoyl CoA α-hydroxylase (PhyH)
    - Requires peroxisomes
- Step 2: Decarboxylation — carbon 1 released as CO_2
- Step 3: Product: Pristanal (1SC) → oxidized to pristanic acid
- Step 4: Pristanic acid-CoA formed → undergoes β-oxidation (in peroxisomes)

2. Refsum Disease

- Cause: Autosomal recessive deficiency of PhyH
- Effect: Accumulation of phytanic acid in plasma and tissues
- Symptoms: Neurologic, e.g., peripheral neuropathy, cerebellar ataxia, retinitis pigmentosa

- Treatment:
    - Strict dietary restriction of phytanic acid (avoidance of green vegetables, ruminant fats, and dairy)

3. ω-Oxidation

- Site: Smooth Endoplasmic Reticulum (SER)
- Process: Oxidation at ω-carbon (methyl end)
    - Produces dicarboxylic acids
- Significance: Normally minor → upregulated in conditions like MCAD deficiency
    - Acts as backup when β-oxidation is impaired

V. Ketone Bodies: AlternativeL Fuel For Cells

- Liver Mitochondrial Role:
    - Converts acetyl CoA (from fatty acid oxidation) into ketone bodies.

- Ketone bodies include:
  - Acetoacetate
  - 3-Hydroxybutyrate (β-hydroxybutyrate)
  - Acetone (nonmetabolized)
  - (Note: Acetoacetate and 3-hydroxybutyrate are functional organic acids.)

- Peripheral Use:
  - Transported via blood to peripheral tissues.
  - Reconverted into acetyl CoA → enters TCA cycle for energy.
  - Tissues that use ketone bodies:
    - Skeletal muscle
    - Cardiac muscle
    - Intestinal mucosa
    - Renal cortex
    - Brain (during prolonged fasting or high ketone levels)

- Advantages of Ketone Bodies:

a. Water-soluble — no need for lipoproteins or albumin.

b. Formed when hepatic acetyl CoA exceeds liver's oxidative capacity.

c. Utilized in proportion to their plasma concentration by extrahepatic tissues.

- Glucose-Sparing Role:
    - Especially important during prolonged fasting.
    - Reduces glucose requirement of brain and peripheral tissues.
- Clinical Note:
    - Fatty acid oxidation disorders:
        - Lead to hypoketosis (↓ acetyl CoA availability).
        - Lead to hypoglycemia (↑ glucose reliance).

A. Ketone Body Synthesis by the Liver: Ketogenesis

- Trigger:
    - Occurs during fasting when liver receives excess FFAs from adipose tissue.

- Resulting Hepatic Changes:
    - ↑ Acetyl CoA (from β-oxidation):
        - Inhibits pyruvate dehydrogenase.
        - Activates pyruvate carboxylase (PC) → OAA → used in gluconeogenesis (not TCA).
        - NADH ↑ → OAA → malate (see p. 124).
        - Low OAA = acetyl CoA diverted to ketogenesis.
    - Other acetyl CoA sources: Catabolism of ketogenic amino acids.

1. HMG CoA Synthesis (Rate-Limiting Step):

- Step 1: Two acetyl CoA molecules → acetoacetyl CoA (via reversal of fatty acid thiolase step).

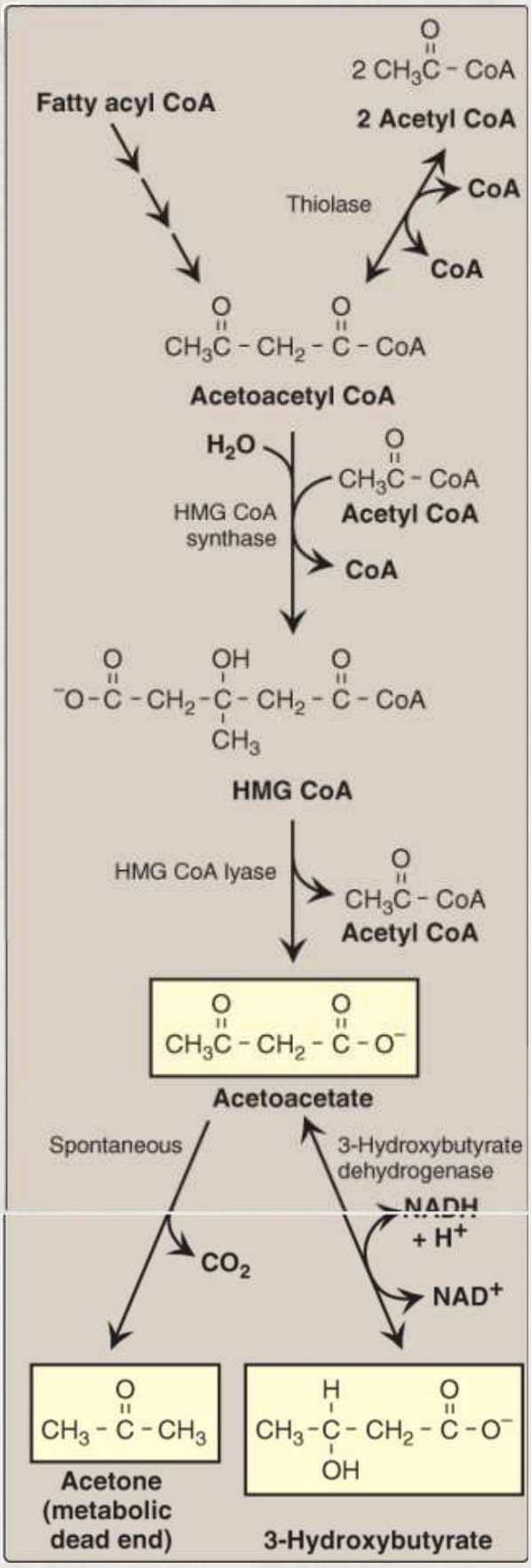
- Step 2: Third acetyl CoA + acetoacetyl CoA → HMG CoA (via HMG CoA synthase).

- HMG CoA synthase:
    - Rate-limiting enzyme of ketogenesis.
    - Found in significant levels only in liver mitochondria.
    - (Note: HMG CoA also appears in cytosolic cholesterol synthesis — different cellular location & context.)

2. Ketone Body Formation:

- HMG CoA → acetoacetate + acetyl CoA (via HMG CoA lyase).
- Acetoacetate can:
    - Be reduced to 3-hydroxybutyrate (using NADH).
    - Spontaneously decarboxylate to acetone in blood.
- Transport:
    - Not CoA-bound → can freely cross mitochondrial membrane.
- Equilibrium between ketones:
    - Controlled by NAD^+/NADH ratio.
    - Fatty acid oxidation → low NAD^+/NADH ratio → favors 3-hydroxybutyrate formation.

Synthesis of Ketone Bodies



B. Ketone Body Use by the Peripheral Tissues: Ketolysis

- Liver constantly produces low levels of ketone bodies.
- During fasting, production increases to supply energy to peripheral tissues.
- Step 1: Oxidation of 3-hydroxybutyrate
    - 3-Hydroxybutyrate → Acetoacetate (via 3-hydroxybutyrate dehydrogenase).
    - NADH is produced in the process.
- Step 2: Activation of acetoacetate
    - Acetoacetate + CoA (from succinyl CoA) → Acetoacetyl CoA
    - Enzyme: Succinyl CoA:acetoacetate CoA transferase (thiophorase)
    - This reaction is reversible, but:
        - Acetoacetyl CoA is rapidly cleaved to two acetyl CoA by thiolase, pulling the reaction forward.

- BUtilization Sites:
    - Extrahepatic tissues, including:
        - Skeletal muscle
        - Cardiac muscle
        - Brain (during prolonged fasting)
    - Excluded tissues:
        - RBCs (lack mitochondria)
        - Liver: despite producing ketone bodies, lacks thiophorase, so cannot use them as fuel.

C. Excessive Ketone Body Production in Diabetes Mellitus

- Overproduction of ketone bodies leads to:
    - Ketonemia (elevated blood ketone levels)
    - Ketonuria (presence in urine)

C. Excessive Ketone Body Production in Diabetes Mellitus

- Overproduction of ketone bodies leads to:
    - Ketonemia (elevated blood ketone levels)
    - Ketonuria (presence in urine)

- Common in uncontrolled Type I Diabetes Mellitus (TID):
    - Blood ketones: can reach 90 mg/dL (normal: <3 mg/dL)
    - Urinary excretion: up to 5,000 mg/24 hrs
- Acidemia Mechanism:
    - Ketone bodies (acetoacetate, 3-hydroxybutyrate) have acidic carboxyl groups (pKa ~4)
    - In blood, they lose H^+, leading to decreased blood pH
    - Termed diabetic ketoacidosis (DKA)
- Compounding Factors:
    - Glucose and ketone loss in urine → dehydration
    - Loss of water → concentration of H^+ in plasma → worsens acidosis

- Signs and Symptoms:
    - Fruity odor of breath (from acetone, a volatile ketone)
    - May occur in:
        - Uncontrolled TID
        - Prolonged fasting
        - Excessive ethanol intake

Ketone Body Synthesis in the Liver and Use in Peripheral Tissues