BB 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic

Exam-1 material

Exam-2 material

Fermentations
Pyruvate
- pyruvate dehydrogenase (ox-decarbox; S-ester)

Krebs' Cycle
- How did he figure it out?
- Overview
- 8 Steps
  - Citrate Synthase (C-C)
  - Aconitase (=, -OH)
  - Isocitrate dehydrogenase (ox-decarbox; =O)
  - Ketoglutarate dehydrogenase (ox-decarbox; S-ester)
  - Succinyl-CoA synthetase (sub-level phos)
  - Succinate dehydrogenase (=)
  - Fumarase (-OH)
  - Malate dehydrogenase (=O)
- Energetics
- Regulation
- Summary

Oxidative Phosphorylation
- Energetics (–0.16 V needed for making ATP)
- Mitochondria
- Transport (2.4 kcal/mol needed to transport H^+ out)
- Electron transport
  - Discovery
  - Four Complexes
    - Complex I: NADH → $CoQH_2$
    - Complex II: Succinate → $CoQH_2$
    - Complex III: $CoQH_2$ → Cytochrome C (Fe^{2+})
    - Complex IV: Cytochrome C (Fe^{2+}) → H_2O
- Chemiosmotic theory: Phosphorylation
  - ATPase
  - Mitchell Hypothesis
  - Binding-Change Model
  - Connection to the proton motive force
  - Net ATP production
  - Regulation

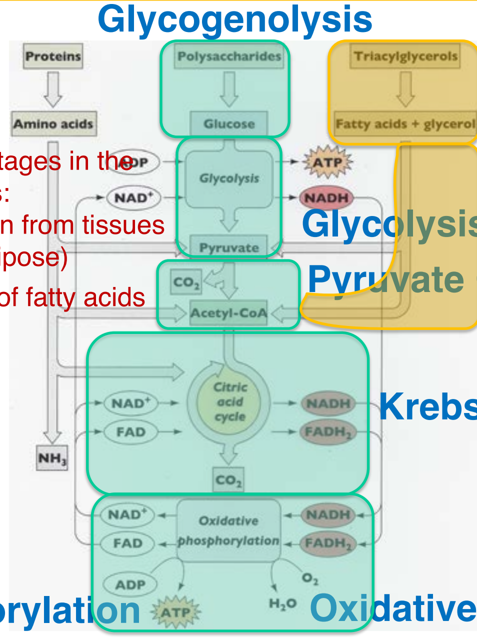
Exam-2 material

Exam-3 material

Catabolism: Lipid Degradation
- Digestion and storage
- FOUR stages lipid catabolism
  - Mobilization from adipose tissues
  - Activation of fatty acids
  - Transport into mitochondria
  - Oxidation
    - Saturated
    - Unsaturated
    - Odd-chain
- Ketone Bodies
- Oxidation in other organelles

Catabolism: Nitrogenous
- Digestion & turnover of proteins
- Urea Cycle
- Amino-acid Degradation

Lipid Degradation: Fatty Acids



There are FOUR stages in the catabolism of lipids:
1) Mobilization from tissues (mostly adipose)
2) Activation of fatty acids
3) Transport
4) Oxidation

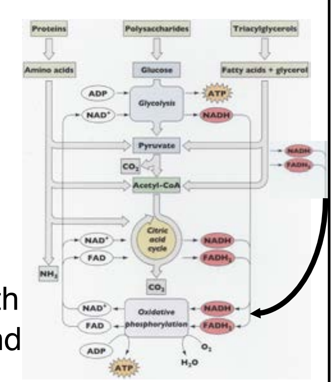
Oxidation

Degradation of Saturated Fatty Acids

Oxidation

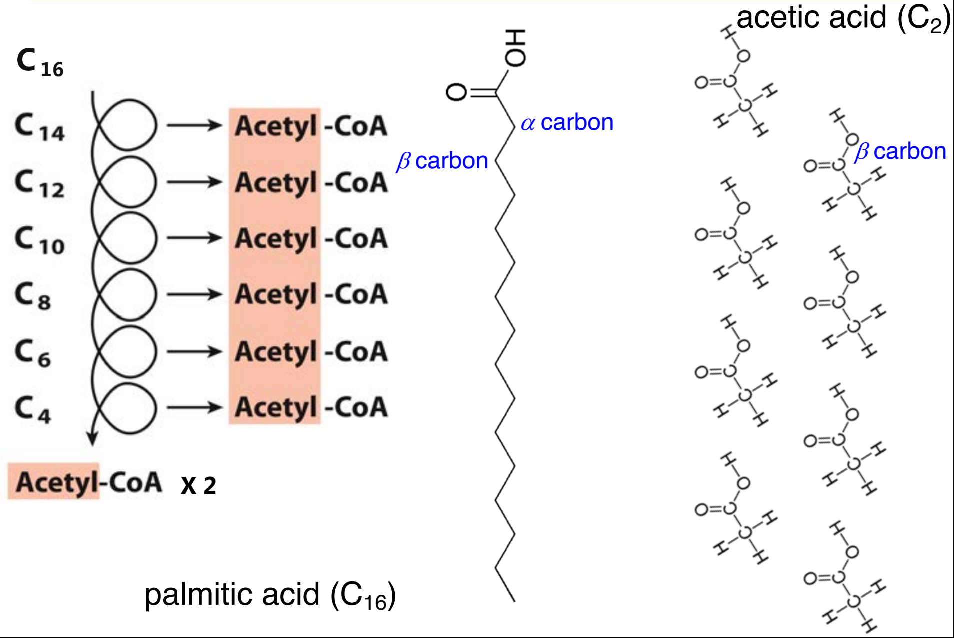
Fatty Acid Degradation

- Fatty Acid Oxidation consists of oxidative conversion of two-carbon units into acetyl-CoA at the β carbon of the fatty acid with concomitant generation of NADH and $FADH_2$.
  - –involves oxidation of β carbon to thioester of fatty acyl-CoA
  - –Concept of thiolysis; similar to phosphorolysis for polysaccharides

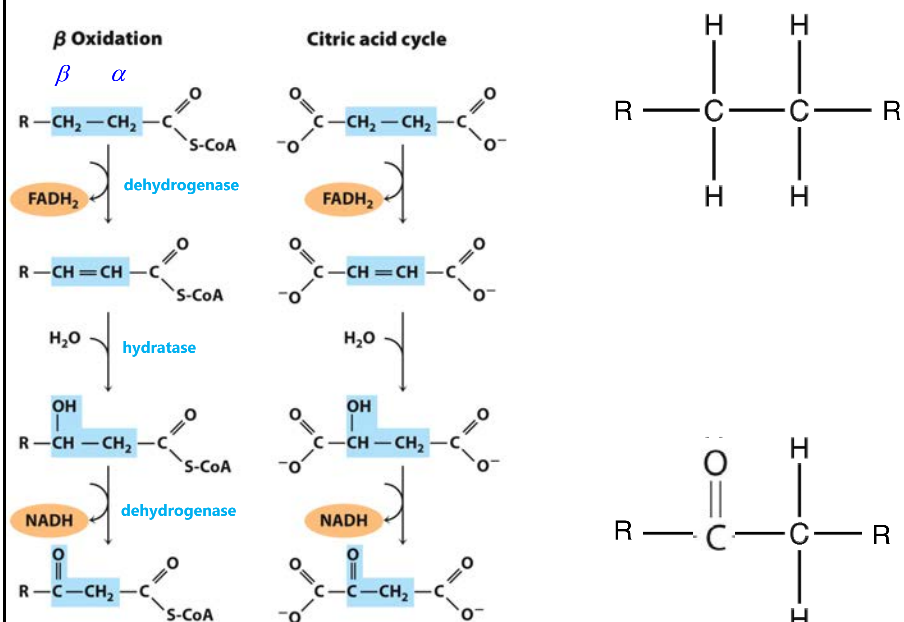


- As we just learned, the acetyl-CoA is converted into CO_2 via citric acid cycle with concomitant generation of more NADH and $FADH_2$. The NADH and $FADH_2$ are re-oxidized via the electron transport down the respiratory chain, and conversion into ATP.
- CONVERGENT PATHWAY with GLUCOSE.

Fatty Acid Degradation

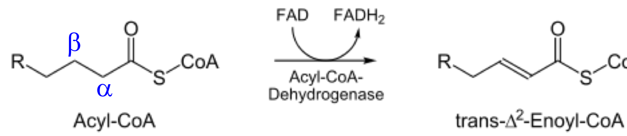


Fatty Acid Degradation

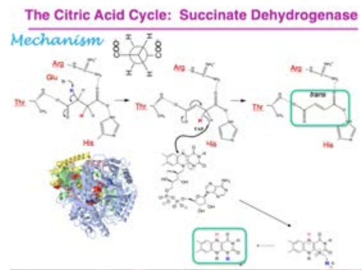


Fatty Acid Degradation

β-Oxidation: Acyl-CoA Dehydrogenase



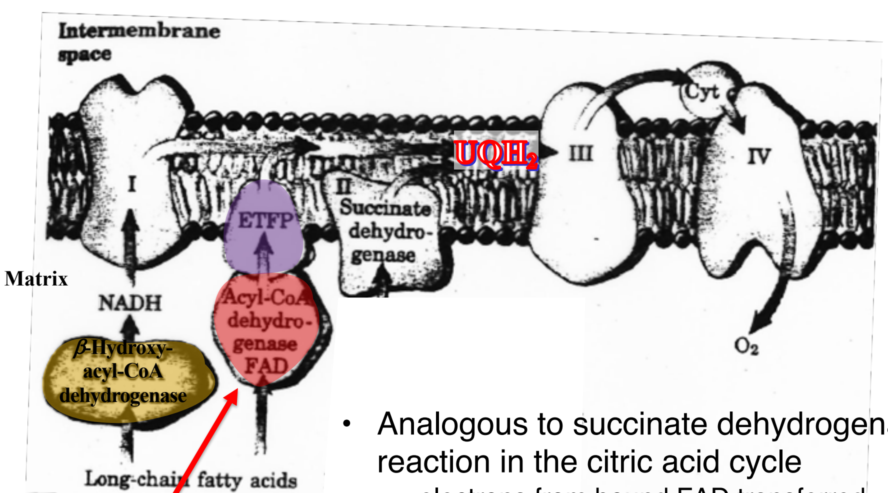
- Catalyzed by isoforms of acyl-CoA dehydrogenase (AD) on the inner-mitochondrial membrane (peripheral)
  - very-long-chain AD (12–18 carbons)*
  - medium-chain AD (4–14 carbons)
  - short-chain AD (4–8 carbons)
- Results in *trans* double bond, different from naturally occurring unsaturated fatty acids
- Mechanism same as succinate dehydrogenase; except not in membrane
- Notice that both α- and β-carbons must be sp^3 to work



*Adrenoleukodystrophy (ALD)

Fatty Acid Degradation

β-Oxidation: Acyl-CoA Dehydrogenase

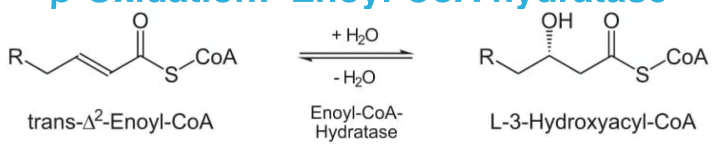


Different isozymes bind to same ETFP

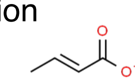
- Analogous to succinate dehydrogenase reaction in the citric acid cycle
  - electrons from bound FAD transferred directly to the electron- transport chain via electron-transferring flavoprotein (ETFP)

Fatty Acid Degradation

β-Oxidation: Enoyl-CoA hydratase



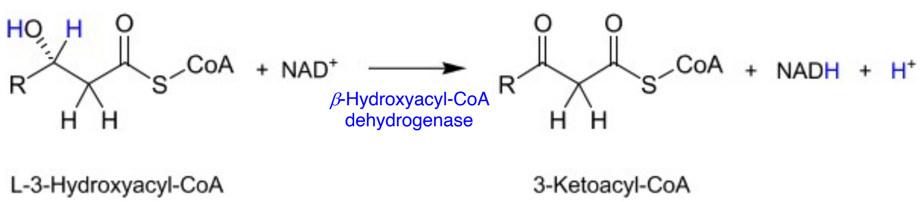
- Catalyzed by two isoforms of enoyl-CoA hydratase:
  - soluble short-chain hydratase (crotonase)
  - membrane-bound long-chain hydratase, part of trifunctional protein (TFP)
- Water adds across the double bond yielding alcohol on β carbon.
- Specific for single *trans*-Δ^2 double bond, no conjugation
- Sometimes called crotonase
- Analogous to fumarase reaction in the citric acid cycle
  - Same stereo-specificity
  - Same enolic intermediate



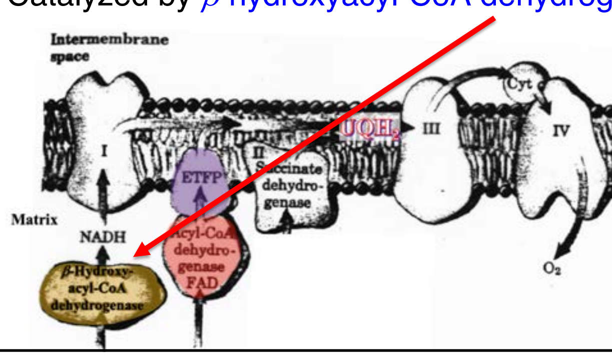
crotonate

Fatty Acid Degradation

β-Oxidation: β-hydroxyacyl-CoA dehydrogenase

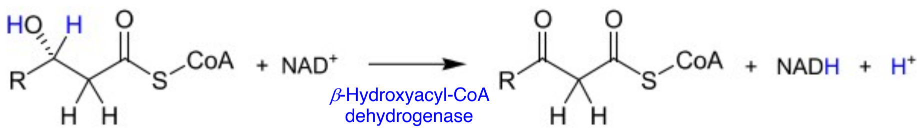


- Catalyzed by β-hydroxyacyl-CoA dehydrogenase



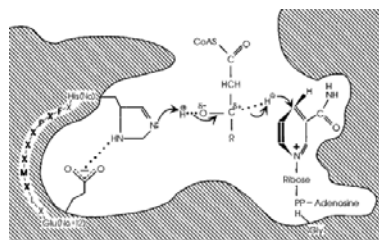
Fatty Acid Degradation

β-Oxidation: β-hydroxyacyl-CoA dehydrogenase



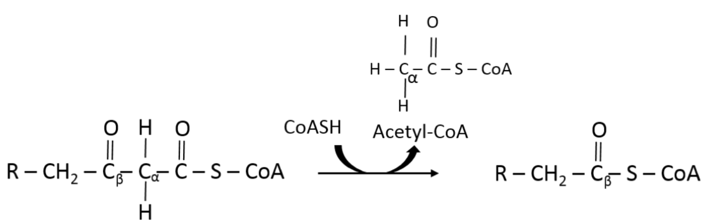
L-3-Hydroxyacyl-CoA 3-Ketoacyl-CoA

- Catalyzed by *β*-hydroxyacyl-CoA dehydrogenase
- The enzyme uses NAD^+ cofactor as the hydride acceptor.
- Only L-isomers of hydroxyacyl CoA act as substrates.
- Analogous to malate dehydrogenase reaction in the citric acid cycle and the lactate & alcohol dehydrogenase in fermentation



Fatty Acid Degradation

β-Oxidation: Thiolase



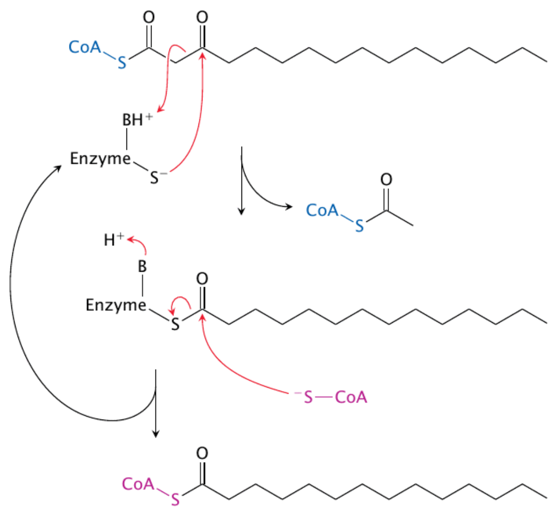
- Catalyzed by acyl-CoA acetyltransferase (thiolase) via covalent mechanism
  - The carbonyl *β*-carbon in *β*-ketoacyl-CoA is electrophilic.
  - Active-site thiolate acts as a nucleophile and releases acetyl-CoA.
  - Terminal sulfur in CoA-SH acts as a nucleophile and picks up the fatty acid chain from the enzyme.
- The net reaction is thio-lysis of the carbon-carbon bond.

Fatty Acid Degradation

Mechanism

β-Oxidation: Thiolase

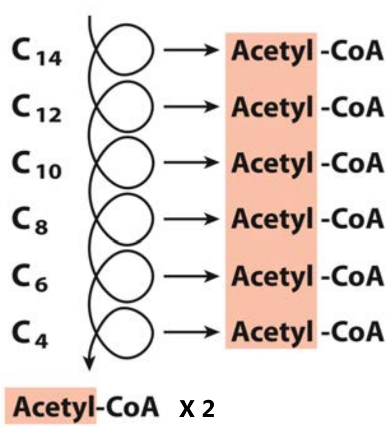
- The carbonyl β-carbon in β-ketoacyl-CoA is electrophilic.
- Active-site thiolate acts as a nucleophile and releases acetyl-CoA.
- Terminal sulfur in CoA-SH acts as a nucleophile and picks up the fatty acid chain from the enzyme.



Fatty Acid Degradation

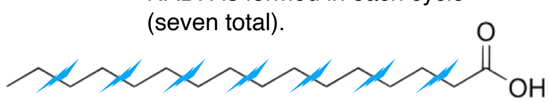
The β-Oxidation Pathway

palmitic acid (C_{16} → C_{14})



How much energy from palmitate?

- For palmitic acid (C_{16})
  - Repeating the previous four-step process six more times (seven total) results in eight molecules of acetyl-CoA.
    - $FADH_2$ is formed in each cycle (seven total).
    - NADH is formed in each cycle (seven total).



- The 8 Acetyl-CoA molecules enters citric acid cycle and further oxidizes into CO_2.
  - This makes more GTP, NADH, and $FADH_2$ (8, 24, and 8, respectively)
- Electrons from all $FADH_2$ (15) and NADH (31) enter the respiratory chain.

Fatty Acid Degradation

Energy from Fatty Acid Catabolism

TABLE 17-1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2O (C16)

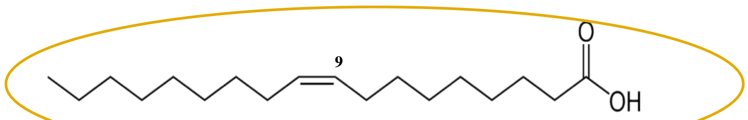
Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed[a]
***β* Oxidation**		
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Citric acid cycle	31	→77.5 ATP
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase 15		8[b]
Succinate dehydrogenase →22.5 ATP	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108 − 2 = 106*

[a]These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.
[b]GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 516).

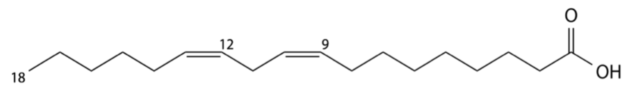
*These 2 ”ATP” equivalents were expended in the activation by Fatty acyl–CoA synthetase.

Degradation of Unsaturated Fatty Acids

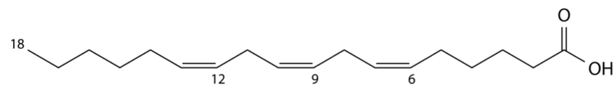
Degradation of Unsaturated Fatty Acids



oleic (C18:1 Δ^9)



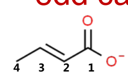
linoleic (C18:2 Δ^9,Δ^{12})



γ-linolenic (C18:3 Δ^6,Δ^9,Δ^{12})

TWO problems:

1. *cis*
2. often at odd carbon

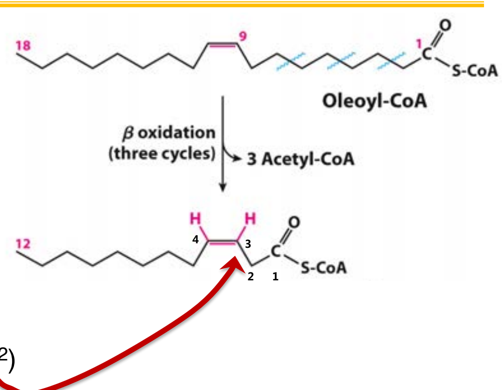


crotonate

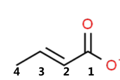
Both linoleic and γ-linolenic (ω6), as well as α-linolenic (ω3; C18:3 Δ^9,Δ^{12},Δ^{15}) are essential fatty acids (EFA)

Fatty Acid Degradation

Oleoyl-CoA (C18:1 Δ^9)

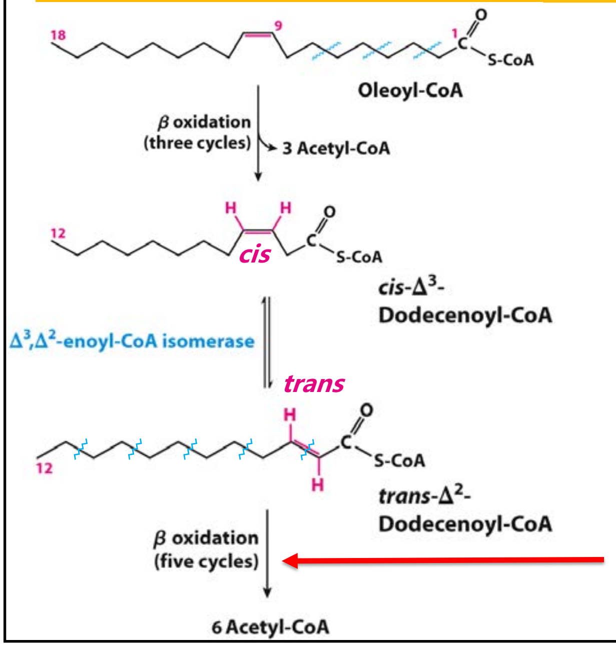


- Naturally occurring unsaturated fatty acids contain *cis* double bonds
  - are NOT a substrate for acyl-CoA dehydrogenase (recall that both α- and β-carbons need to be sp3)
  - Bond is *cis* and Δ^3 (C3 is sp^2)
- One additional enzyme is required for this:
  - Δ^2,Δ^3-enoyl-CoA isomerase: converts *cis* double bonds starting at carbon 3 to trans double bonds at carbon 2
- Mono-unsaturated fatty acids at odd carbons require only the isomerase.
- You lose an $FADH_2$ equivalent from first step of β-oxidation due to double bond……….



crotonate

Fatty Acid Degradation

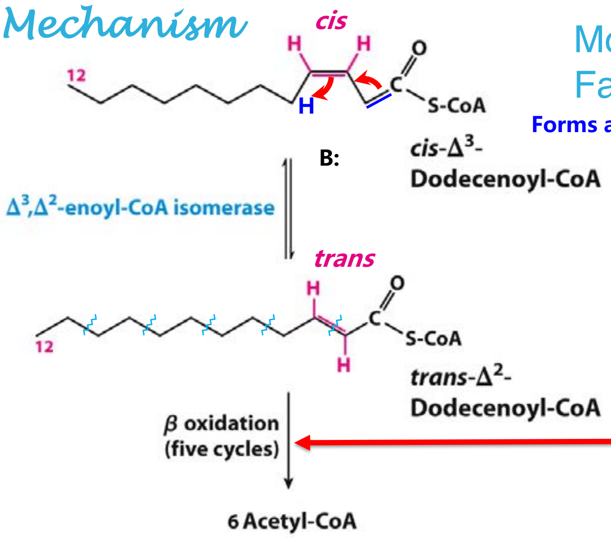


Monounsaturated Fatty Acids

During first of five remaining cycles, acyl-CoA dehydrogenase step is skipped, resulting in 1 fewer $FADH_2$.

Fatty Acid Degradation

Mechanism



Monounsaturated Fatty Acids

Forms an enolic intemediate

The first step of five remaining cycles, acyl-CoA dehydrogenase is skipped, resulting in 1 fewer $FADH_2$.

Fatty Acid Degradation

Energy from Fatty Acid Catabolism

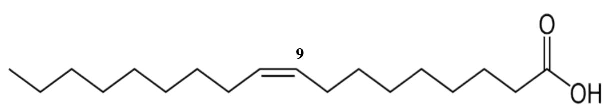
TABLE 17-1a Yield of ATP during Oxidation of One Molecule of Oleoyl-CoA (C18) to CO_2 and H_2O

Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed[a]
***β* Oxidation**		
Acyl-CoA dehydrogenase (8-1=7; 1 lost due to isomerase)	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	8 NADH	20
Citric acid cycle	35	→87.5 ATP
Isocitrate dehydrogenase	9 NADH	22.5
α-Ketoglutarate dehydrogenase	9 NADH	22.5
Succinyl-CoA synthetase 16		9[b]
Succinate dehydrogenase →24 ATP	9 $FADH_2$	13.5
Malate dehydrogenase	9 NADH	22.5
Total		120.5 – 2 = 118.5*

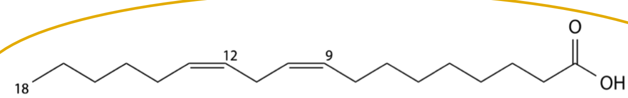
[a]These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.
[b]GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 516).

*These 2 "ATP" equivalents were expended in the activation by Fatty acyl–CoA synthetase.

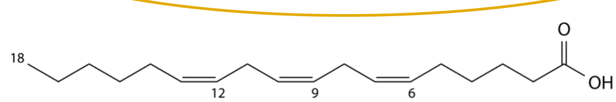
Degradation of Unsaturated Fatty Acids



oleic (C18:1 Δ^9)

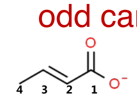


linoleic (C18:2 Δ^9,Δ^{12})



γ-linolenic (C18:3 Δ^6,Δ^9,Δ^{12})

TWO problems:
1. *cis*
2. often at odd carbon



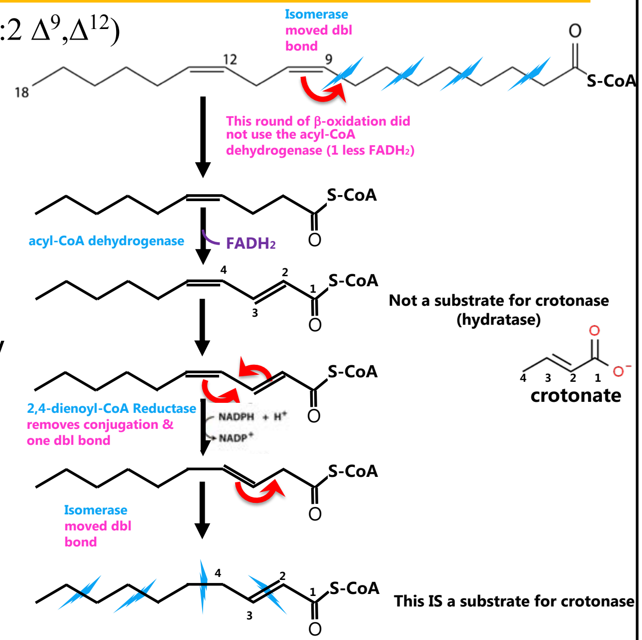
crotonate

Both linoleic and γ-linolenic (ω6), as well as α-linolenic (ω3; C18:3 Δ^9,Δ^{12},Δ^{15}) are essential fatty acids (EFA)

Fatty Acid Degradation

Linoleoyl-CoA (C18:2 Δ^9,Δ^{12})

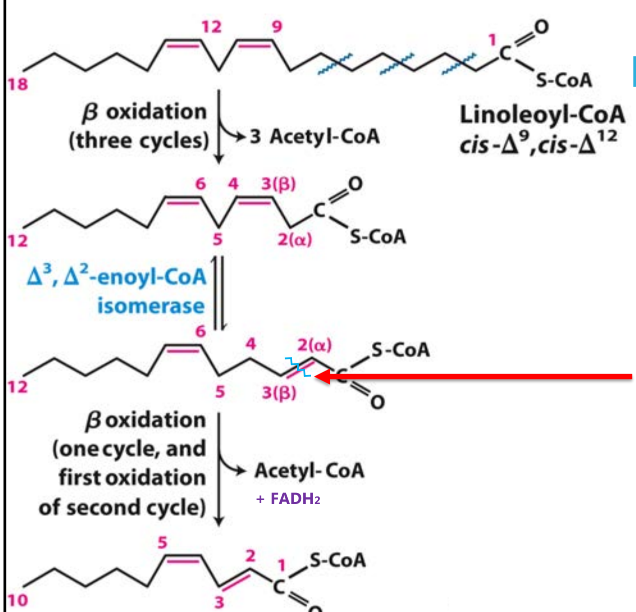
- Another additional enzyme is required for cis double bonds not at carbon 3:
  - reductase: reduces cis double bonds at Δ^4
- Polyunsaturated fatty acids require both enzymes.
- Remember at the first dbl-bond you lose 1 $FADH_2$ equivalent from first step of β-oxidation and use the isomerase instead & at the second dbl-bond you use an NADPH (NADH) for the reductase.



Fatty Acid Degradation

Oxidation of Polyunsaturated Fatty Acids

Isomerization



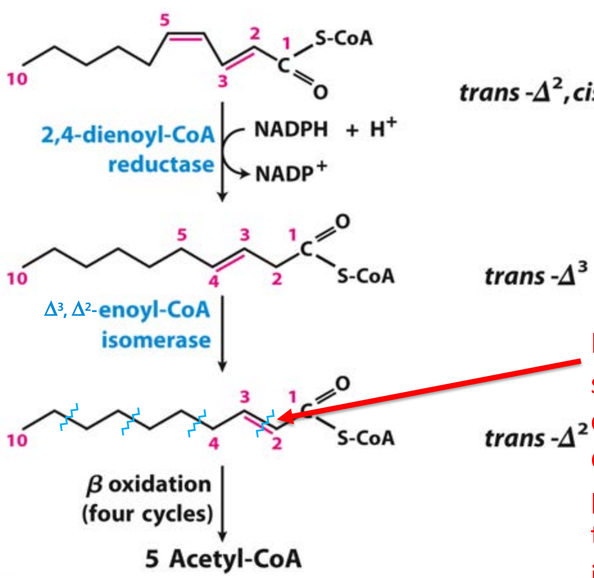
During this β-oxidation, acyl-CoA dehydrogenase step is skipped, resulting in 1 fewer $FADH_2$.

This conjugated situation is not a substrate for the hydratase

Fatty Acid Degradation

Oxidation of Polyunsaturated Fatty Acids

Reductase



Recall that during first step of the four remaining cycles, the acyl-CoA dehydrogenase already put in the dbl-bond prior to reductase and isomerase reactions.

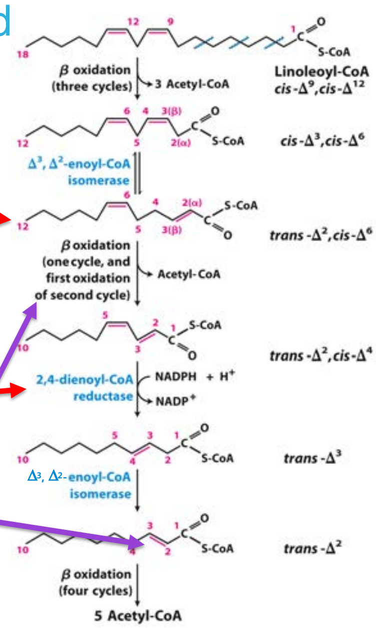
Fatty Acid Degradation

Oxidation of Polyunsaturated Fatty Acids

Results in 1 fewer $FADH_2$ after isomerization, as the acyl-CoA dehydrogenase step is skipped and goes right to the hydratase.

NADPH reduces the 2 conjugated unsaturated bonds to one trans-double bond between them; but its still at an odd carbon

This bond already made with $FADH_2$ already generated before reductase and isomerization.



Fatty Acid Degradation

Energy from Fatty Acid Catabolism

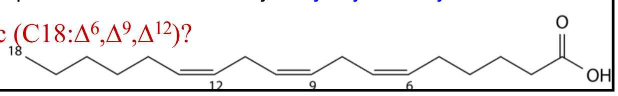
TABLE 17-1a Yield of ATP during Oxidation of One Molecule of Linoleoyl-CoA to CO_2 and H_2O (C18)

Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed[a]
β Oxidation		
Acyl-CoA dehydrogenase (8-1=7; 1 lost due to isomerase)	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase (8-1=7; 1 used for reductase)	7 NADH	17.5
Citric acid cycle	34	→85 ATP
Isocitrate dehydrogenase	9 NADH	22.5
α-Ketoglutarate dehydrogenase	9 NADH	22.5
Succinyl-CoA synthetase 16		9[b]
Succinate dehydrogenase →24 ATP	9 $FADH_2$	13.5
Malate dehydrogenase	9 NADH	22.5
Total		118 – 2 = 116*

[a]These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.
[b]GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 516).

*These 2 "ATP" equivalents were expended in the activation by Fatty acyl–CoA synthetase.

What would it be for γ-linolenic (C18:Δ^6,Δ^9,Δ^{12})?



Degradation of Odd-Chain Fatty Acids

Fatty Acid Degradation

Oxidation of Odd-Numbered Fatty Acids

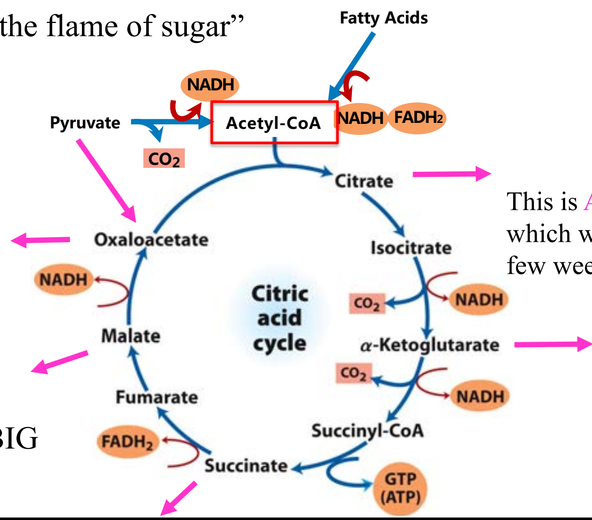
- Most dietary fatty acids are even-numbered.
- Many plants and some marine organisms also synthesize odd-numbered fatty acids.
- The metabolism of oxidation of odd-chain fatty acids CONVERGES with that of some amino acids
- Details will be discussed with amino acids

Ketone Bodies

Ketone Body Degradation

What are Ketone Bodies?

"Fat burns in the flame of sugar"



This is ANABOLISM, which we'll discuss in a few weeks

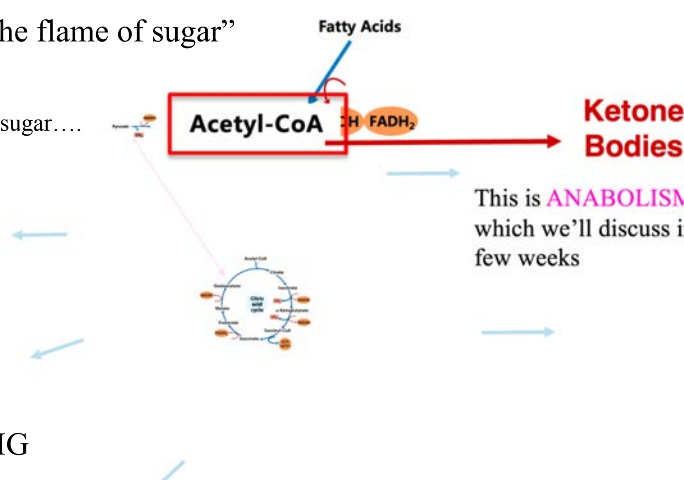
THIS IS A BIG DEAL!!!!

Ketone Body Degradation

What are Ketone Bodies?

"Fat burns in the flame of sugar"

When there is no sugar….



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