Lipid Metabolism, part 2

1

Summary of fatty acid synthesis

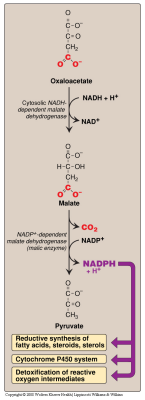
8 acetyl CoA + 14 NADPH + 14 H+ + 7 ATP

palmitic acid (16:0) + 8 CoA + 14 $NADP^+$ + 7 ADP + 7 Pi + 7 H20

1. The major suppliers of NADPH for fatty acid synthesis are:

   a) the **hexose monophosphate shunt**

   b) cytoplasmic **malate dehydrogenase**



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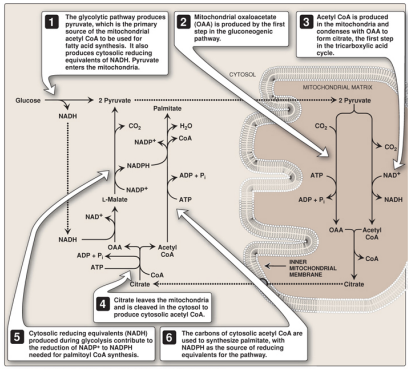
Summary of fatty acid synthesis



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1. Additional two-carbon units can be added to palmitate by separate enzyme systems contained in the ER and mitochondria.
2. Certain cell types in the brain can add up to a total of 24 carbon units to an acyl chain
3. Enzymes present in the ER (mixed-function oxidases) are responsible for desaturating fatty acids using NADPH as a cofactor

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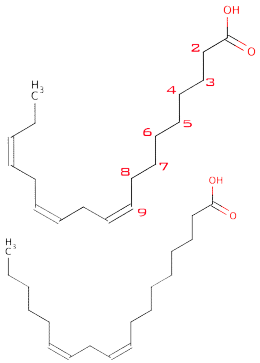
1. Humans do not have the enzymes required to introduce double bonds past the number 9 carbon of fatty acids.
2. Therefore, linoleic and linolenic acids, both important precursor molecules, are considered essential fatty acids



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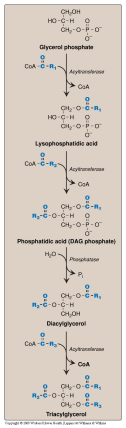
Synthesized fatty acids can be stored as TAG's

1. The fatty acid chains must be activated by fatty acyl CoA synthetases.
   a) This enzyme is located on the outer mitochondrial membrane.
   b) It utilizes ATP to form an acyl adenylate intermediate. Cleavage of the resulting pyrophosphate makes the reaction irreversible
2. Glycolytic intermediates must be tapped to produce glycerol phosphate (the liver (only) can also do this via glycerol kinase.
3. Acyltransferases can build TAG's from activated fatty acids and glycerol phosphate.



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1: Br J Cancer. 1979 Jun;39(6):681-7.

Related Articles, Links

Lipogenetic and glycolytic enzyme activities in carcinoma and nonmalignant diseases of the human breast.

Szutowicz A, Kwiatkowski J, Angielski S.

Activities of some enzymes associated with carbohydrate and lipid metabolism were determined in 48 human breast carcinomas and compared with those found in 35 nonmalignant breast tumours and also in 13 normal breast tissues. In fibrocystic disease only the activity of citrate lyase was markedly higher (14-fold) than in normal tissue. The activities of the remaining enzymes did not differ significantly from those in normal tissue. Enzyme activities in breast carcinoma were 4--160 x those determined in normal tissue according to the following sequence : phosphofructokinase less than malate NADP dehydrogenase less than hexokinase less than lactate dehydrogenase less than isocitrate NADP dehydrogenase less than ATP citrate lyase. Activity of citrate lyase, very low in normal breast (0.0017 mumol/min/g of tissue) rose gradually to 0.039, 0.072 and 0.258 mumol/min/g of tissue in localized fibrocystic disease, fibroadenomas and carcinomas respectively. These data support the idea that citrate lyase may play an important role in lipogenesis in hyperplastic human breast tissues.

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Medical Sciences

Fatty acid synthesis: A potential selective target for antineoplastic therapy

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Communicated by Victor A. McKusick, March 22, 1994

ABSTRACT **OA-519 is a prognostic molecule found in tumor cells from breast cancer patients with markedly worsened prognosis. We purified OA-519 from human breast carcinoma cells, obtained its peptide sequence, and unambiguously identified it as fatty acid synthase through sequence homology and enzymology. Tumor fatty acid synthase is an ~270-kDa polypeptide which specifically abolished immunostaining of human breast cancers by anti-OA-519 antibodies. Tumor fatty acid synthase oxidized NADPH in a malonyl-CoA-dependent fashion and synthesized fatty acids composed of 80% palmitate, 10% myristate, and 10% stearate from acetyl-CoA, malonyl-CoA, and NADPH with a specific activity of 624 nmol of NADPH oxidized per min per mg. Tumor cell lines with elevated fatty acid synthase showed commensurate increases in incorporation of [U-^{14}C]acetate into acylglycerols demonstrating that fatty acid synthase increases occur in the context of overall increases in endogenous fatty acid synthesis. Cerulenin inhibited acylglycerol synthesis in tumor cells and fibroblast controls in a dose-dependent fashion and also caused a growth inhibition which generally paralleled the level of endogenous fatty acid synthesis. Supraphysiologic levels of palmitate, 14 μM in dimethyl sulfoxide, significantly reversed the growth inhibition caused by cerulenin at concentrations of up to 5 μg/ml, indicating that cerulenin-mediated growth inhibition was due to fatty acid synthase inhibition. markedly elevated FAS activity in aggressive tumors may provide a highly selective basis for anticancer therapy.**

METHODS

Purification of OA-519. A lysate of ZR-75-1 cells prepared by Dounce homogenization at 1.5 × 10^6 cells per ml in lysis buffer (20 mM Tris·HCl, pH 7.5 at 4°C/1 mM EDTA/0.1 mM diisopropyl fluorophosphate/0.1 mM phenylmethanesulfonyl fluoride) was centrifuged at 16,000 × *g* for 30 min at 4°C. After passing through a 0.45-μM filter, the lysate was applied to a Sephacryl S-200 (Pharmacia) gel filtration column (2.5 cm × 90 cm) equilibrated in lysis buffer at pH 8.0 at 4°C supplemented with 100 mM KCl and 1 mM 2-mercaptoethanol. Fractions containing protein immunoreactive with polyclonal anti-OA-519 peptide antibody as judged by Western blot analysis of a 4% SDS/polyacrylamide gel were pooled, diluted with an equal volume of lysis buffer without KCl, and loaded onto a Mono Q HR 5/5 anion-exchange column (Pharmacia). The column was washed for 15 min at 1 ml/min, and bound material was eluted with a linear 60-ml gradient over 60 min to 1 M KCl. Fractions containing the immunoreactive ≈270-kDa protein as shown by Western blot were pooled. This procedure results in substantially pure preparations (>95%) of FAS (OA-519) as judged by Coomassie-stained gels.

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Nature Reviews Cancer 7, 763-777 (October 2007) | doi:10.1038/nrc2222

Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis

Javier A. Menendez1 (#a1) & Ruth Lupu2 (#a2). About the authors (/nrc/journal/v7/n10/authors/nrc2222.html)

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There is a renewed interest in the ultimate role of fatty acid synthase (FASN) — a key lipogenic enzyme catalysing the terminal steps in the *de novo* biogenesis of fatty acids — in cancer pathogenesis. Tumour-associated FASN, by conferring growth and survival advantages rather than functioning as an anabolic energy-storage pathway, appears to necessarily accompany the natural history of most human cancers. A recent identification of cross-talk between FASN and well-established cancer-controlling networks begins to delineate the oncogenic nature of FASN-driven lipogenesis. FASN, a nearly-universal druggable target in many human carcinomas and their precursor lesions, offers new therapeutic opportunities for metabolically treating and preventing cancer.

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β-Oxidation of Fatty Acids

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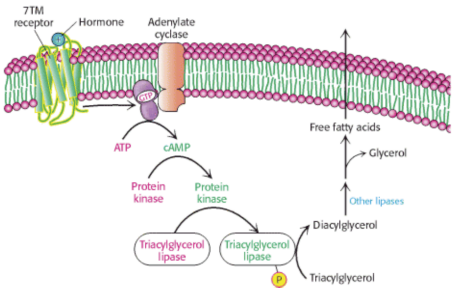
Triacylglycerols are hydrolyzed by cyclic AMP-regulated lipases



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Fatty acids must be esterified to Coenzyme A before they can:

1. undergo oxidative degradation,
2. be utilized for synthesis of complex lipids (e.g., triacylglycerols or membrane lipids),
3. or be attached to proteins as lipid anchors.

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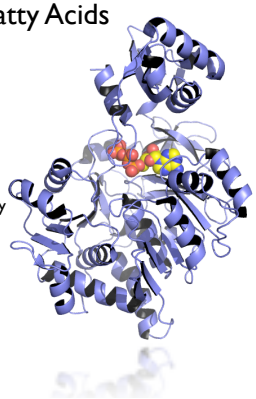
Acyl-CoA-Synthase Catalyzes the Activation of Fatty Acids



1. **Acyl CoA synthetase** catalyzes the activation of a fatty acid in two steps:
   a) It catalyzes the reaction of the fatty acid with ATP to form an acyl adenylate.
   b) Subsequently, it catalyzes the attack by CoA on the acyl adenylate to form acyl-CoA and AMP.
2. Acyl CoA synthetase resides primarily along the **outer mitochondrial membrane**

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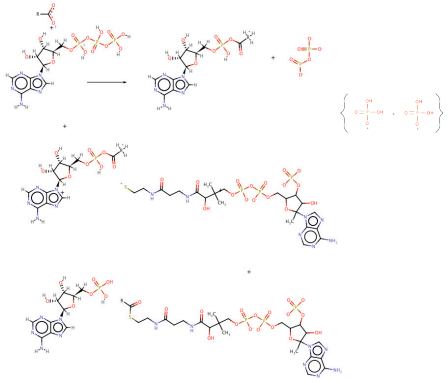

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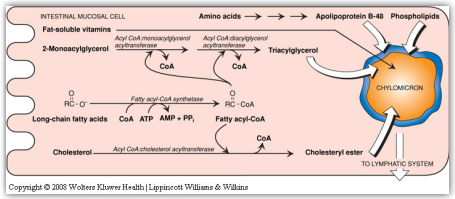
Acyl-CoA-synthase is required for re-synthesis of triacylglycerides and cholesteryl esters



1. Fatty acids are activated by fatty **acyl CoA synthetase** [requires ATP].
2. **Triacylglycerol synthase** re-joins 2-monoacylglycerol with two fatty acyl CoA
3. Cholesterol is re-esterified with fatty acyl CoA by Acyl CoA cholesterol acyltransferase

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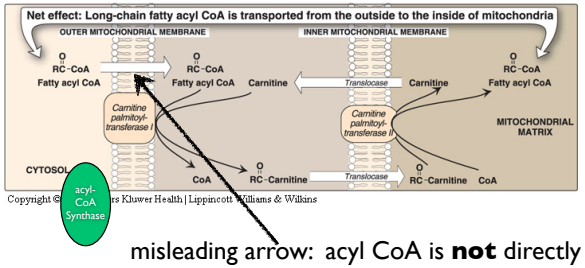
β-oxidation of fatty acids occurs within mitochondria



misleading arrow: acyl CoA is **not** directly shared between the cytoplasm and matrix

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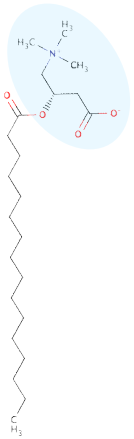
The **carnitine shuttle** brings activated fatty acids into the matrix



1. **carnitine acyl transferase I** replaces the CoA with carnitine
   (a) Note that the cytoplasmic and matrix pools of CoA do not directly mix.
2. **translocases** transfer acyl carnitine into the matrix
3. **carnitine acyl transferase II** swaps the carnitine for CoA
4. carnitine is transferred back to cytoplasm

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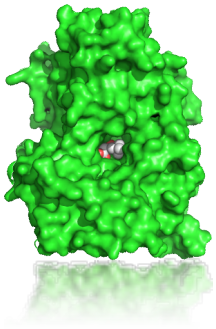
carnitine acyltransferase I



1. associated with outer mitochondrial membrane
2. transfers acyl chain from CoA to carnitine
3. releases CoA and acyl carnitine
4. bears a tunnel that sequesters the acyl chain during catalysis

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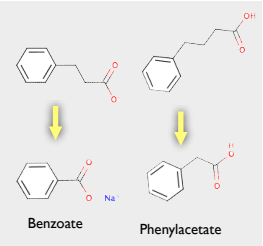
Fatty acids are oxidized two carbon units at a time.

In **1904**, Franz Knoop fed his dog either even or odd-numbered fatty acids labeled with ω-phenyl groups.

Odd-numbered chains always yielded Benzoate in the dog's urine, while even-numbered chains always yielded phenylacetate.

This landmark work was the first to use a synthetic label in an experiment.



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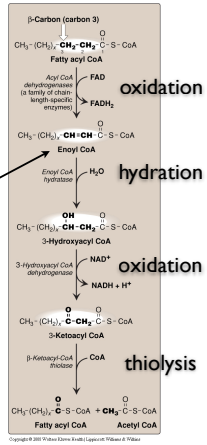
β-oxidation pathway occurs in the mitochondrial matrix.

trans double-bond



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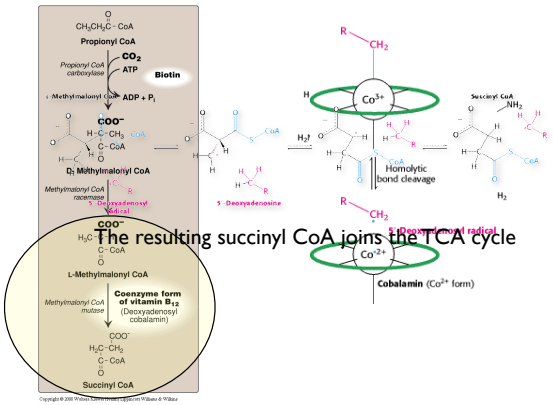
What about odd chain-length fatty acids?



The resulting succinyl CoA joins the TCA cycle

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What about very long fatty acids?

1. Fatty acids that have 20 or more carbon units get chopped into smaller pieces, no smaller than 8 units long, within Peroxisomes.
2. The smaller chains are delivered to the mitochondria where they undergo β-oxidation.

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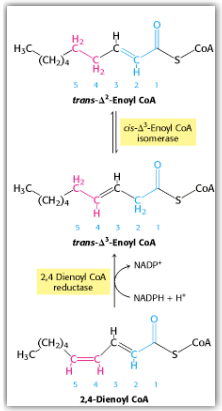
And what about (cis) unsaturated fatty acids?

Two additional enzymes are utilized:

1. **isomerase** (example at right) "moves" dbl bond to appropriate position.
2. **reductase** deals with adjacent double-bonds.



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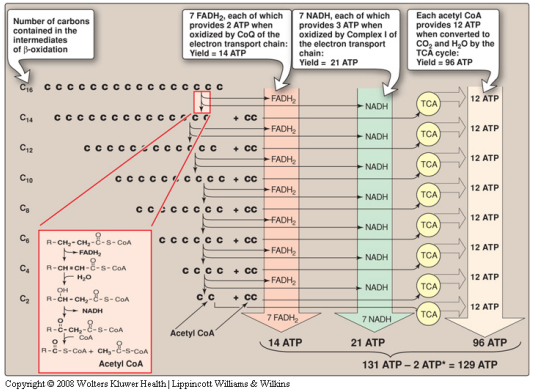

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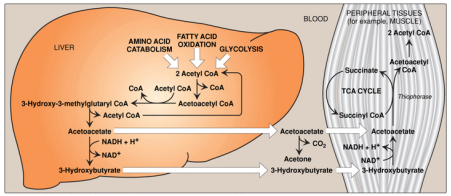
Summary of mitochondrial β-oxidation of fatty acids

	SYNTHESIS	DEGRADATION
Major tissue site	Primarily liver	Muscle, liver
Subcellular location	Primarily cytosol	Primarily mitochondria
Carriers of acyl/acetyl groups between mitochondria and cytosol	Citrate (mitochondria to cytosol)	Carnitine (cytosol to mitochondria)
Phosphopantetheine-containing active carriers	Acyl carrier protein domain, coenzyme A	Coenzyme A
Oxidation/reduction coenzymers	NADPH (reduction)	NAD^+, FAD (oxidation)
Two-carbon donor/product	Malonyl CoA: donor of one acetyl group	Acetyl CoA: product of β-oxidation
Activator	Citrate	
Inhibitor	Long-chain fatty acyl CoA (inhibits *acetyl CoA carboxylase*)	Malonyl CoA (inhibits *carnitine palmitoyltransferase-I*)
Product of pathway	Palmitate	Acetyl CoA
Repetitive four-step process	Condensation, reduction dehydration, reduction	Dehydrogenation, hydration dehydrogenation, thiolysis

Synthesis of ketone bodies by the liver



1. During fasting, oxaloacetate is diverted to gluconeogenesis and hence is unavailable to the TCA cycle.
2. Acetyl-CoA is then diverted from the TCA cycle and condensed into acetoacetyl CoA and, finally, acetoacetate
3. Acetoacetate can be transported to peripheral tissues and converted to two acetyl-CoA