MILK BIOSYNTHESIS

PART 3: FAT
FDPase = fructose diphosphatase

- Citrate lyase
- Isocitrate dehydrogenase
- Fatty acid synthetase
- Acetyl CoA carboxylase
- Fatty acyl deacylase – thioesterase II
- Lipoprotein lipase
MILK FAT TRIGLYCERIDES

- Synthesized on the smooth endoplasmic reticulum and form small droplets

- Numerous small lipid droplets will fuse together as the growing lipid droplet moves toward the apical membrane

- At the apical membrane, the large lipid droplet forces out the apical membrane of the cell, the apical membrane surrounds the lipid droplet until it pinches off and enters the lumen

- Milk fat/lipid globule now surrounded by a membrane (originally part of cell's apical membrane)
WHERE DO THE MILK DROPLETS COME FROM?

- Milk fat triglycerides are synthesized in the mammary epithelial cells
- Fatty acids used to synthesize milk triglycerides may arise from two sources:
  - Breakdown of blood lipids
  - De novo synthesis within the mammary epithelial cells
40 to 60% of milk fatty acids come from blood

Mostly from very low density lipoproteins (VLDL)
  - Synthesized in intestines and liver

VLDL are 90 to 95% lipid on inside and 5 to 10% protein on outer surface

Triglycerides in the VLDL are hydrolyzed in the mammary capillaries by lipoprotein lipase (LPL)
LPL can hydrolyze off one, two or all three of the fatty acids from the glycerol backbone.

- Results in free fatty acids plus diacylglycerides, monoacylglycerides, or glycerol.

- Free fatty acids, monoacylglycerides, diacylglycerides and glycerol can be taken up by epithelial cells and be reused for triglyceride synthesis.
DE NOVO SYNTHESIS

- Fat synthesis within the mammary gland (starting from scratch)

- Acetate is the main carbon source
  - β-hydroxybutyrate (BHBA) can also serve as the initial 4 carbons
  - Both absorbed through cell’s basolateral membrane

- Fatty acids are built 2 carbons at a time
  - 16 carbon limit
DE NOVO FATTY ACID SYNTHESIS

- Synthesis of short and medium chain fatty acids in the mammary gland occurs this way.
- Occurs in the cytoplasm of the mammary epithelial cell.
- In ruminants, the carbon sources used for FA synthesis are acetate and BHBA.
- Glucose is the carbon source for FA synthesis in non-ruminants.
# Proportional Contribution of Sources of Fatty Acids in Cow Milk

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>% of FA from De novo synthesis</th>
<th>% of FA from VLDL Fatty Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4 - C10</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>C12</td>
<td>80-90</td>
<td>10-20</td>
</tr>
<tr>
<td>C14</td>
<td>30-40</td>
<td>60-70</td>
</tr>
<tr>
<td>C16</td>
<td>20-30</td>
<td>70-80</td>
</tr>
<tr>
<td>C18</td>
<td>0</td>
<td>100</td>
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</tbody>
</table>
The Fatty Acid Synthesis Pathway involves the following steps:

- Activation - acetyl-CoA carboxylation
- Elongation - the malonyl-CoA pathway
- Condensation step
- Reduction step
- Dehydration step
- Another reduction step
- The cycle is then repeated

The malonyl-CoA pathway occurs with the growing FA chain esterified to an acyl carrier protein.
Each cycle through the malonyl-CoA pathway results in two carbons being added to the FA chain.

Total reaction is (e.g. palmitate; C16):

- Acetyl-CoA + 7 Malonyl-CoA + 14 NADPH\(_2\) are catalyzed by Fatty Acid Synthetase to yield = Palmitate + 7 CO\(_2\) + 14 NADP + 8 CoA
Fatty acid synthetase is a large complex of enzymatic activities which are responsible for the reactions of FA synthesis.

Acylthioesterases cleave off the growing FA chain from the acyl carrier protein once it has reached a certain chain length.

Medium chain acylthioesterase cleaves off the growing FA chain at or before it reaches C16.

- In nonruminants, medium chain acylthioesterase is cytoplasmic and cleaves off free FAs.
- In ruminants, medium chain acylthioesterase is associated with the fatty acid synthetase complex and releases acyl-CoA thioesters.
FATTY ACID SYNTHESIS

- Below is a diagram of the pathway of fatty acid synthesis
- Acetate carbons come in twice
  - Source of acetyl-CoA to enter the malonyl-CoA pathway
  - Source of malonyl-CoA that adds the two carbons to each cycle of the FA synthetase
- Conversion of acetyl-CoA to malonyl-CoA is the rate limiting step in FA synthesis
- Reaction is catalyzed by acetyl-CoA carboxylase
- Acetyl-CoA carboxylase activity is regulated by lactogenic hormones and is one of the enzymes up-regulated during the first stage of lactogenesis
MALONYL COA PATHWAY

- In the Malonyl-CoA Pathway, the condensation step is the covalent linking of acetyl-CoA and Malonyl-CoA

- First reduction step is the conversion of acetoacetyl-ACP to β-hydroxybutryl-ACP

- Dehydration step is the conversion of β-hydroxybutryl-ACP to crotonyl-ACP

- Second reduction step is the conversion of crotonyl-ACP to butryl-ACP

- Butryl-ACP condenses with another malonyl-CoA to start the second cycle.

- Even though malonyl-CoA is a three carbon primer, one carbon is lost in the condensation step and therefore only two carbons are added to the growing fatty acid chain at each round
Acetate\[\text{CH}_3\text{C-OH}\] + CoA + ATP \rightarrow Acetyl-CoA\[\text{CH}_3\text{C-CoA}\]

Acetyl-CoA + CO$_2$ + ATP \rightarrow Malonyl-CoA\[\text{CH}_2\text{C-CoA}\]

Malonyl-CoA \rightarrow Acetyl-CoA

Malonyl-CoA + Acetyl-CoA \rightarrow Butyl-ACP

Butyl-ACP \rightarrow Butyl-CoA

Butyl-CoA + CO$_2$ \rightarrow Acetyl-CoA

Acetyl-CoA + HO$-$ \rightarrow BHBA

*ACP = acyl Carrier Protein
β-HYDROXYBUTYRATE

- Abbreviated BHBA
- Can enter cycle as a primer only
- Cannot be used in fatty acid synthesis at later stages
- Contributes up to 50% of the first 4 carbons
- Cannot be split into acetate in the cytosol, but can be converted to 2 acetyl-CoA's in the mitochondria
  - Can’t leave the mitochondria = not available for FA synthesis
In ruminants, dietary and carbohydrates fats are generally metabolized in the rumen so that the primary source of carbons for FA synthesis by the mammary gland are acetate and BHBA.

Glucose is limiting in ruminants.

Absence of citrate lyase in ruminants means that little glucose carbons end up being used for FA synthesis.

Glucose is required for generation of reducing equivalents in ruminants and nonruminants.
RUMINANT VS. NON-RUMINANT FATTY ACID SYNTHESIS

- NADPH$_2$ supplies the necessary reducing equivalents for the Fatty Acid Synthesis Pathway

- Acetyl-CoA carboxylase is a key milk fat synthesis enzyme activity which increases during lactogenesis

- Close relationship between observed FA synthesis by mammary tissue and activity of acetyl-CoA carboxylase during lactogenesis and lactation
- **Acetyl CoA carboxylase**
  - Acetyl-CoA + HCO3- + ATP → malonyl-CoA + ADP + Pi

- **Fatty acid synthase**
  - Acetyl-CoA + 7 malonyl-CoA + 14 (NADPH + H+) → Palmitic acid (16 carbons) + 7 CO2 + 14 NADP + 8 CoA + 6 H2O
- Acetate and β-hydroxybutyrate are primers
  - Acetyl-CoA and butyryl-CoA synthase
- Addition of malonyl-CoA all from acetate
- Glucose does not contribute to carbons of fatty acids in ruminants
  - Lack citrate lyase
NON-RUMINANTS

- 1. NADP malate dehydrogenase
- 2. Citrate lyase
- 3. Acetyl-CoA carboxylase
- 4. Fatty acid synthase
RUMINANTS

1. Isocitrate dehydrogenase
2. Acetyl-CoA synthase
3. Butyryl-CoA synthase
4. Acetyl-CoA carboxylase
5. Fatty acid synthase
GLUCOSE

PENTOSE PHOSPHATE CYCLE

Fructose-6-P

Triose-P

NADPH

α-Glycerol-P

NADH

NADH

NADH

NADPH

FATTY ACIDS

Malonyl CoA

Malate

Pyruvate

Citrate

Oxaloacetate

Acetyl CoA

TRICARBOXYLIC ACID CYCLE

BHBA

Acetyl CoA

ACETATE

x = cows can't use citrate via this pathway.

MITOCHONDRIA

ACYTOSE

CYTOSOL
KEY ENZYMES

- **Acetyl-CoA carboxylase**
  - Rate limiting enzyme for the fatty acid synthesis pathway

- **Fatty acid synthetase**
  - Large complex of enzyme activities responsible for the chain elongation of the fatty acid

- **Fatty acyl deacylase – thioesterase II**
  - In liver and adipose tissue, fatty acid synthesis is terminated when there are > 16 carbons by a thioesterase I
  - In epithelial cells of rats, mice, and rabbits, thioesterase II terminates synthesis after the addition of 8 to 14 carbons
FATTY ACID SYNTHESIS SUMMARY

- Occurs in cytoplasm
- Intermediates are linked to acyl carrier protein
- Enzymes of fatty acid synthesis are linked in a complex
- Elongation occurs by 2 carbons/cycle
  - Source of 2-carbon units = acetyl-CoA via Malonyl CoA
    - Malonyl CoA actually contributes the carbons each pass through the cycle
- Required reducing agent = NADPH₂
- Elongation stops at C16
- Required for de novo fatty acid synthesis:
  - Carbon source (acetyl-CoA)
  - Source of reducing equivalents (NADPH₂)
PREFORMED FATTY ACIDS: NEFA

- Released from adipose tissue by hormone-sensitive lipase during periods of energy shortage
- Travel in blood via albumin
- Only significant during first month of lactation
- Activated to fatty acyl-CoA
FATTY ACID SYNTHESIS VIDEO

- https://www.youtube.com/watch?v=3HFnXtsgV78
GLUCOSE SPARING MECHANISMS IN RUMINANTS

- When nutrients are restricted, body tissues are generally mobilized (fat > muscle > bone)
- Underfed cows can mobilize about 15% of their body protein, with muscle protein contributing approximately half of total body protein loss
- Plasma NEFA (non-esterified fatty acids) are used as an energy source by maternal and fetal tissues
  - Also enriches milk fat content
  - Elevated NEFA can depress feed intake and suppress the immune system
Metabolic Adaptation (Schoenberg, 2010)

- Increased mobilization of fatty acids
- Decreased insulin response of adipose tissue
- Decreased uptake of glucose by peripheral tissues
- Impaired glucose disposal
- Increased hepatic gluconeogenesis

Changes in intake

Glucose sparing for gravid uterus and eventually the mammary gland
Some degree of fat mobilization is normal

Excessive fat mobilization (elevated NEFA) associated with metabolic disorders, lower milk production, and poor reproductive performance

Excessive insulin resistance in body fat likely contributes to hypermobilization of NEFA and lower DMI (resemble Type II diabetics)
  - Fat cows
  - Cows overfed energy during dry period
Figure 2. Metabolic and endocrine adaptations to undernutrition in the ruminant (AA, amino acids; NEFA, non-esterified fatty acids; VFA, volatile fatty acids; T3-T4, thyroid hormones).
GLUCOSE SPARING MECHANISMS IN RUMINANTS

- 70% of glucose taken up used for lactose synthesis
- Propionate used for blood glucose production
- Acetate used for milk FA and energy
- Absence of citrate cleavage enzyme
  - Prevents use of glucose for milk FA
- Pentose phosphate pathway

⭐ Recycling of 3-C units allows more efficient production of NADPH
QUESTIONS?

IF THIS IS WHAT 1% BODY FAT LOOKS LIKE, I'D HATE TO SEE WHAT I'D LOOK LIKE IF I LET MYSELF GO.