MILK BIOSYNTHESIS

PART 3: FAT

KEY ENZYMES (FROM ALL BIOSYNTHESIS LECTURES)

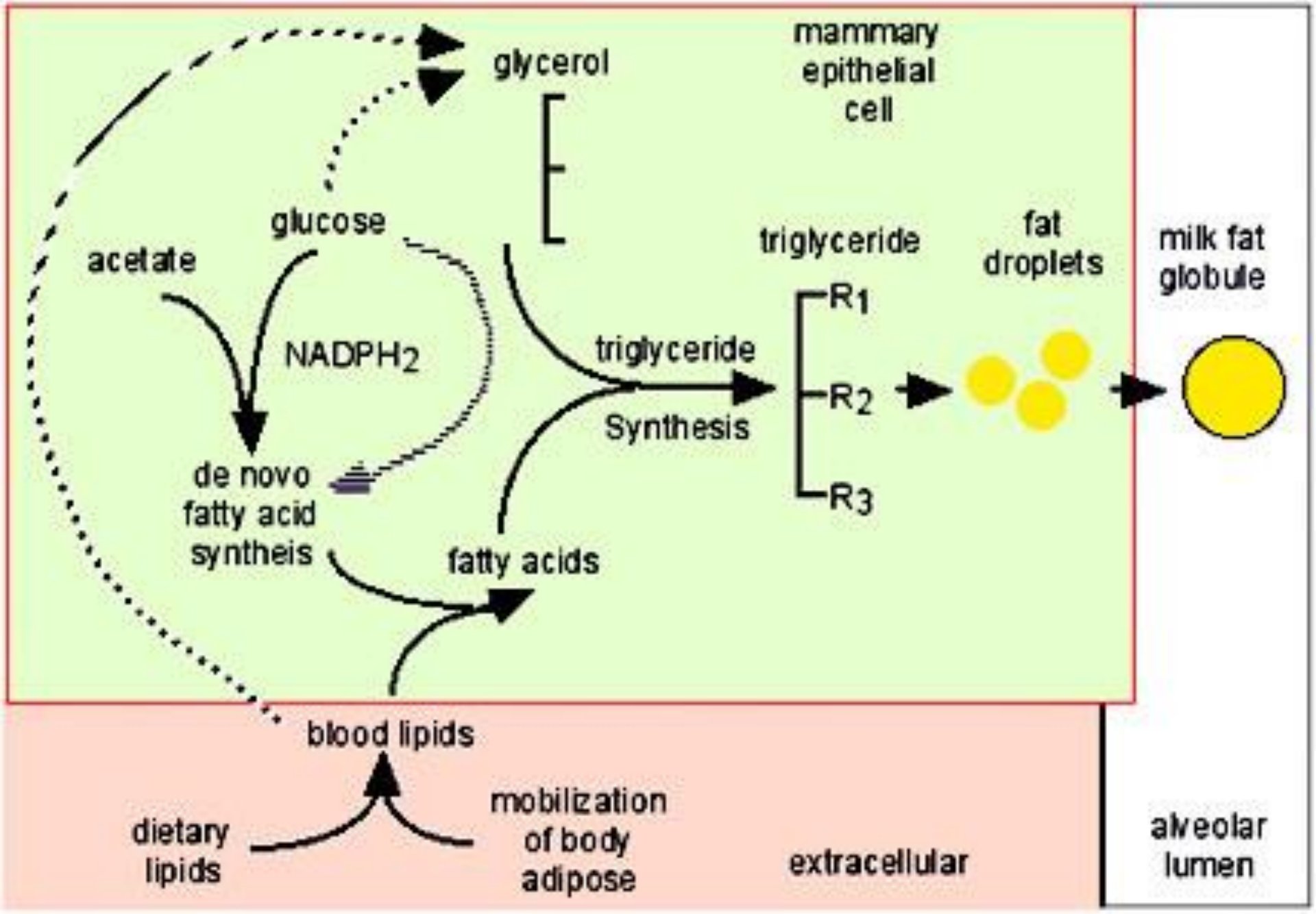
- ☑ FDPase = fructose diphosphatase
- Citrate lyase
- Isocitrate dehydrogenase
- Fatty acid synthetase
- Acetyl CoA carboxylase
- Fatty acyl deacylase – thioesterase II
- Lipoprotein lipase

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


mammary epithelial cell
glycerol
glucose
acetate
NADPH2
de novo fatty acid syntheis
fatty acids
triglyceride Synthesis
triglyceride
R1
R2
R3
fat droplets
milk fat globule
blood lipids
dietary lipids
mobilization of body adipose
extracellular
alveolar lumen

BLOOD LIPIDS

- 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

- 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, monacylglycerides, diacylgycerides and glycerol can be taken up by epithelial cells and be reused for triglyceride synthesis

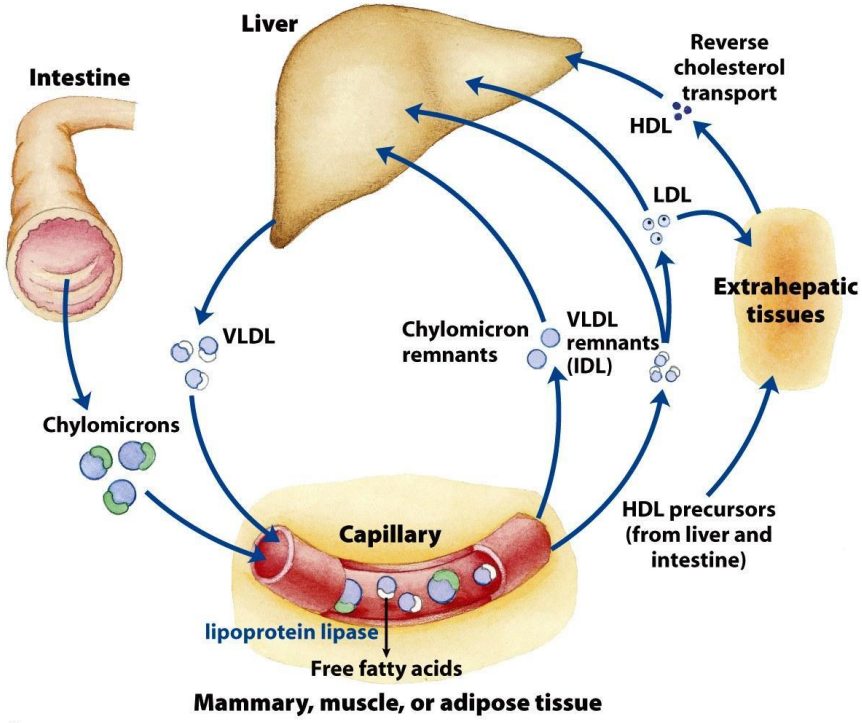


Figure 21-40a
Lehninger Principles of Biochemistry, Fifth Edition

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

Fatty Acid	% of FA from De novo synthesis	% of FA from VLDL Fatty Acid
C4 -C10	100	0
C12	80-90	10-20
C14	30-40	60-70
C16	20-30	70-80
C18	0	100

FATTY ACID SYNTHESIS PATHWAY

- 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

MALONYL-COA PATHWAY

- 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

ENZYMES IN FATTY ACID SYNTHESIS PATHWAY

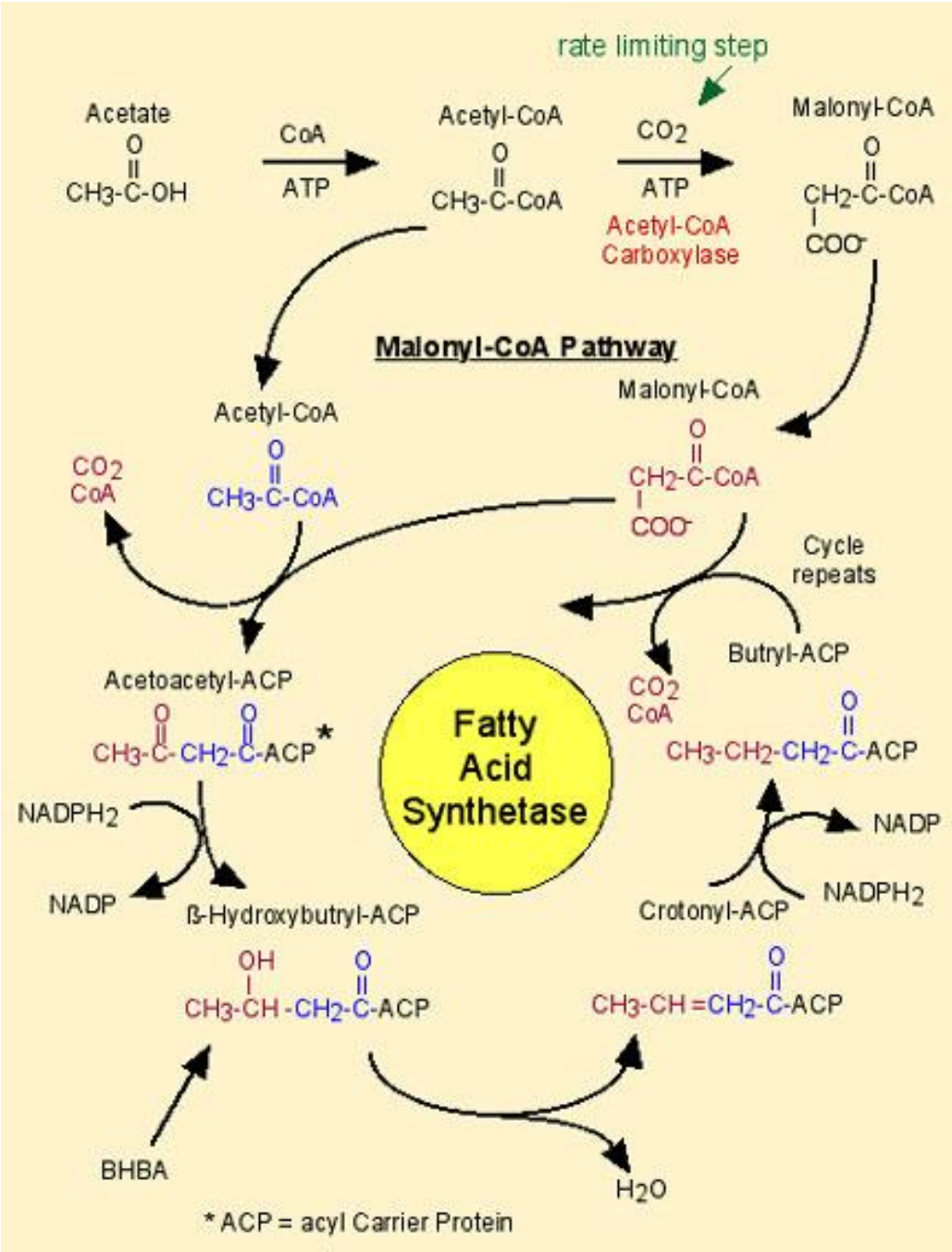
- Fatty acid synthetase is a large complex of enzymatic activities which are responsible for the reactions of FA synthesis.
- Acylthioesterases cleave off the growing FA chain from the acyl carrier protein once it has reached a certain chain length
- Medium chain acylthioesterase cleaves off the growing 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 he 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 ß-hydroxybutryl-ACP
- Dehydration step is the conversion of ß-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


rate limiting step
Acetate
CH3-C-OH
CoA
ATP
Acetyl-CoA
CH3-C-CoA
CO2
ATP
Acetyl-CoA Carboxylase
Malonyl-CoA
CH2-C-CoA
COO-
Malonyl-CoA Pathway
Acetyl-CoA
CO2
CoA
CH3-C-CoA
Malonyl-CoA
CH2-C-CoA
COO-
Cycle repeats
Butryl-ACP
Acetoacetyl-ACP
CH3-C-CH2-C-ACP*
Fatty Acid Synthetase
CO2
CoA
CH3-CH2-CH2-C-ACP
NADPH2
NADP
NADP
NADPH2
ß-Hydroxybutryl-ACP
Crotonyl-ACP
OH
CH3-CH-CH2-C-ACP
CH3-CH=CH2-C-ACP
BHBA
H2O
* ACP = acyl Carrier Protein

ß-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

RUMINANT VS. NON-RUMINANT FATTY ACID 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

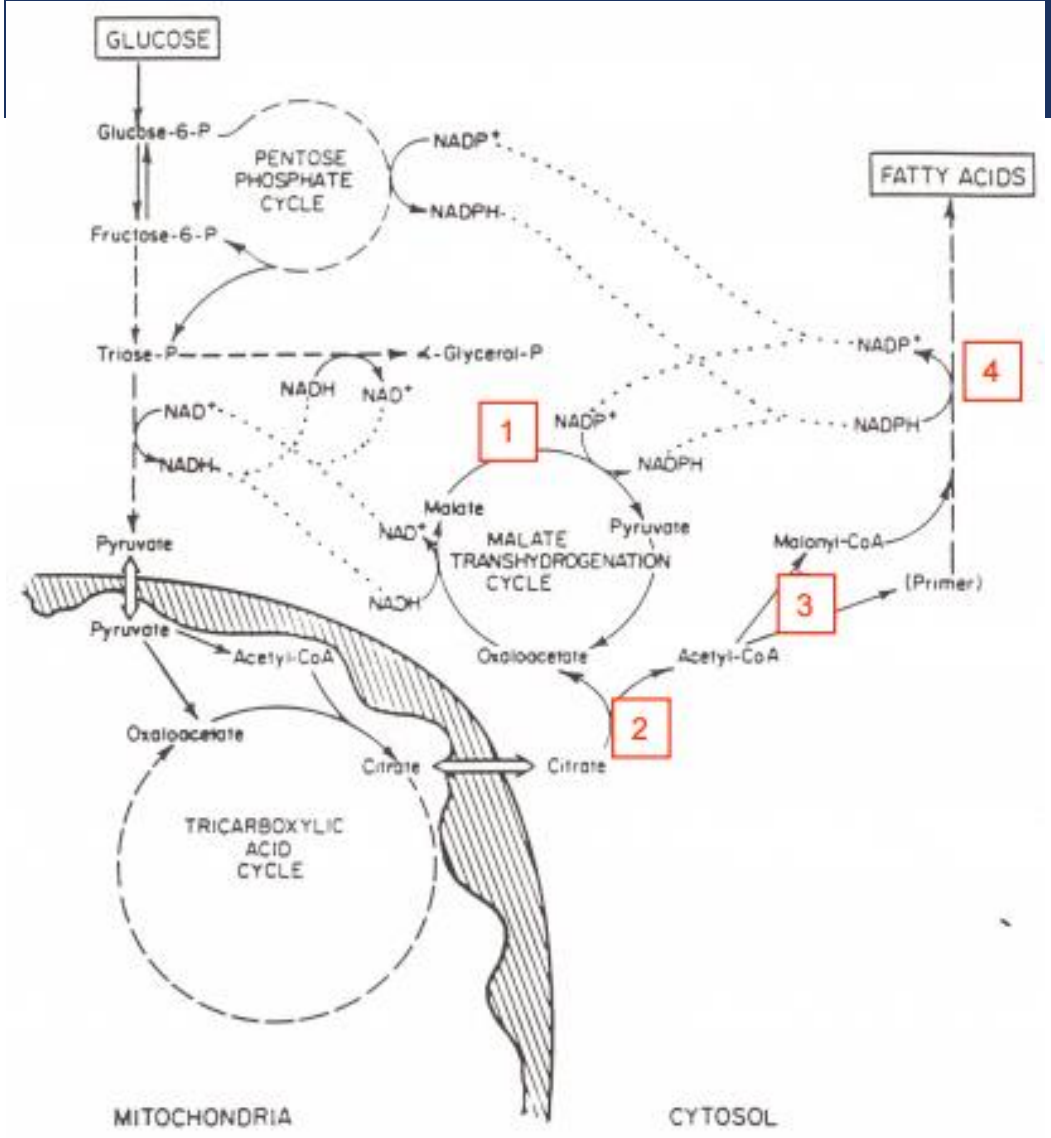
- $NADPH_2$ supplies the necessary reducing equivalents for the Fatty Acid Synthesis Pathway
- Acetyl-CoA carboxylase is a key milk fat synthesis enzyme activity which increases during lactogenesis
- Close relationship between observed FA synthesis by mammary tissue and activity of acetyl-CoA carboxylase during lactogenesis and lactation

- Acetyl CoA carboxylase
  - Acetyl-CoA + HCO_3^- + ATP → malonyl-CoA + ADP + Pi

- Fatty acid synthase
  - Acetyl-CoA + 7 malonyl-CoA + 14 (NADPH + H+) → Palmitic acid (16 carbons) + 7 CO_2 + 14 NADP + 8 CoA + 6 H_2O

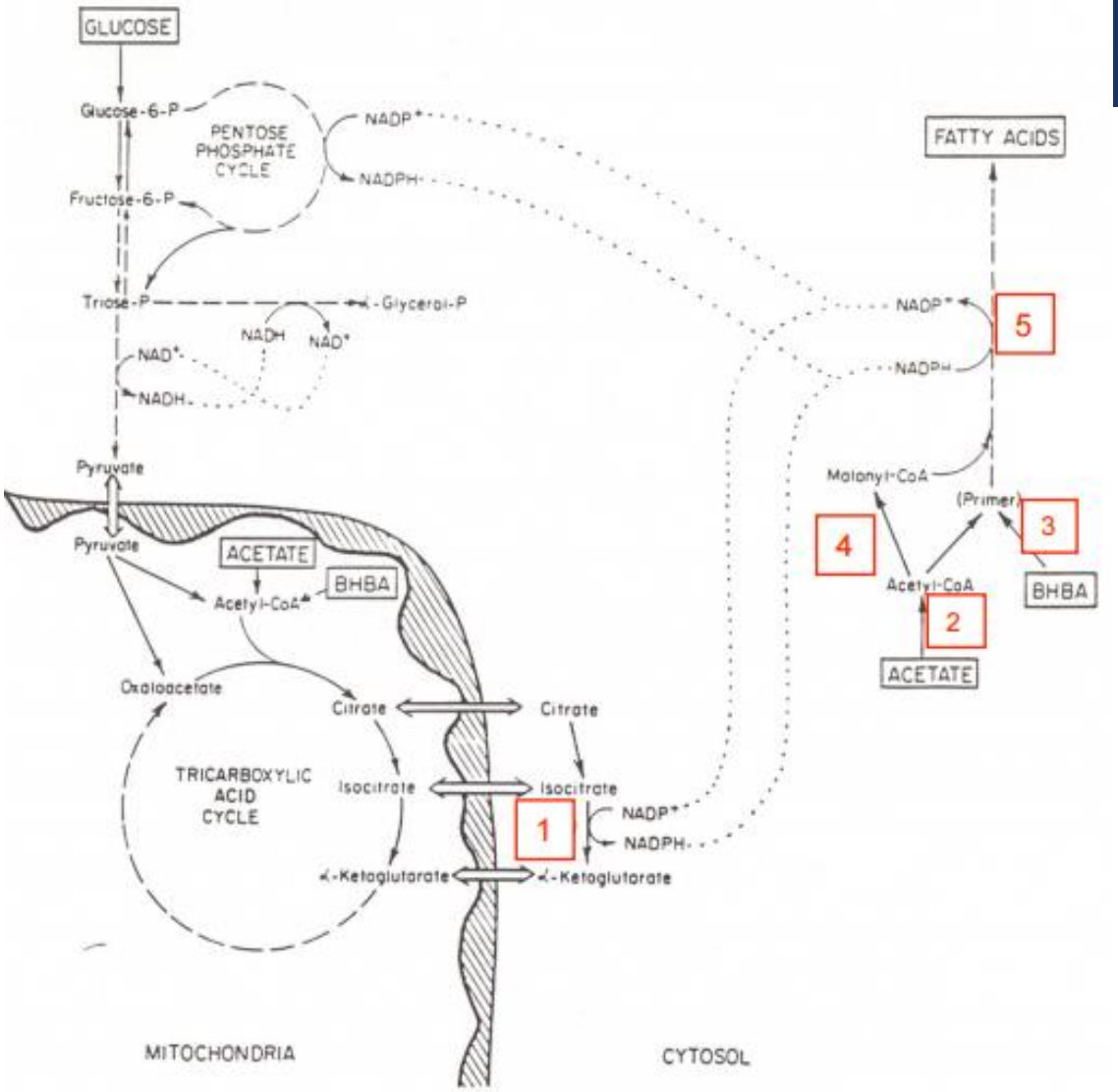
- Acetate and β-hydroxybutyrate are primers
  - Acetyl-CoA and butyryl-CoA synthase
- Addition of malonyl-CoA all from acetate
- Glucose does not contribute to carbons of fatty acids in ruminants
  - Lack citrate lyase

NON-RUMINANTS

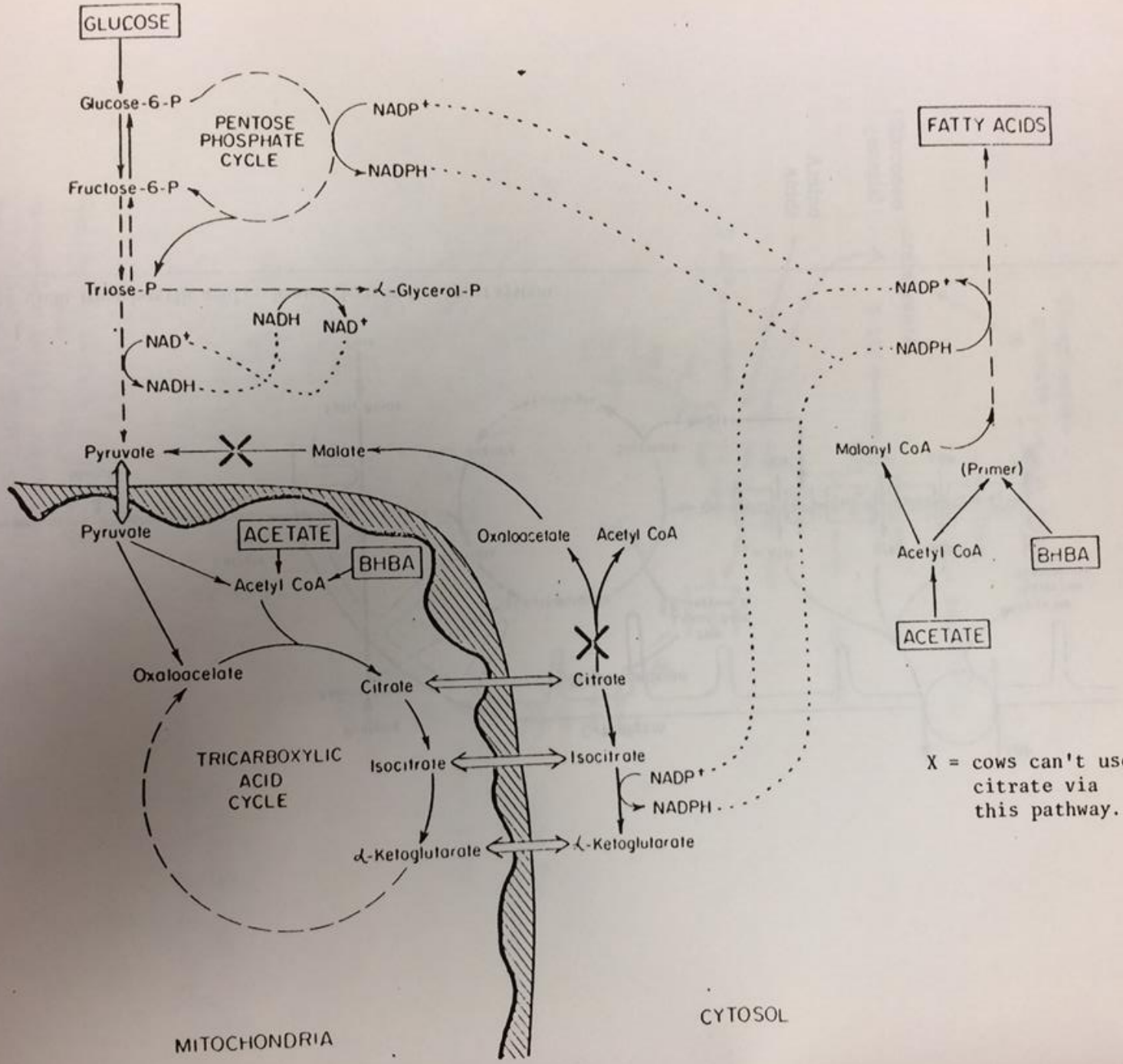


- 1. NADP malate dehydrogenase
- 2. Citrate lyase
- 3. Acetyl-CoA carboxylase
- 4. Fatty acid synthase

RUMINANTS



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


GLUCOSE
Glucose-6-P
PENTOSE PHOSPHATE CYCLE
NADP+
NADPH
Fructose-6-P
FATTY ACIDS
Triose-P
α-Glycerol-P
NADH
NAD+
NAD+
NADH
NADP+
NADPH
Pyruvate
Malate
Malonyl CoA
(Primer)
Pyruvate
ACETATE
BHBA
Acetyl CoA
Oxaloacetate
Acetyl CoA
Acetyl CoA
BHBA
ACETATE
Oxaloacetate
Citrate
Citrate
TRICARBOXYLIC ACID CYCLE
Isocitrate
Isocitrate
NADP+
NADPH
α-Ketoglutarate
α-Ketoglutarate
X = cows can't use citrate via this pathway.
MITOCHONDRIA
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 = $NADPH_2$
- Elongation stops at C16
- Required for de novo fatty acid synthesis:
  - Carbon source (acetyl-CoA)
  - Source of reducing equivalents ($NADPH_2$)

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

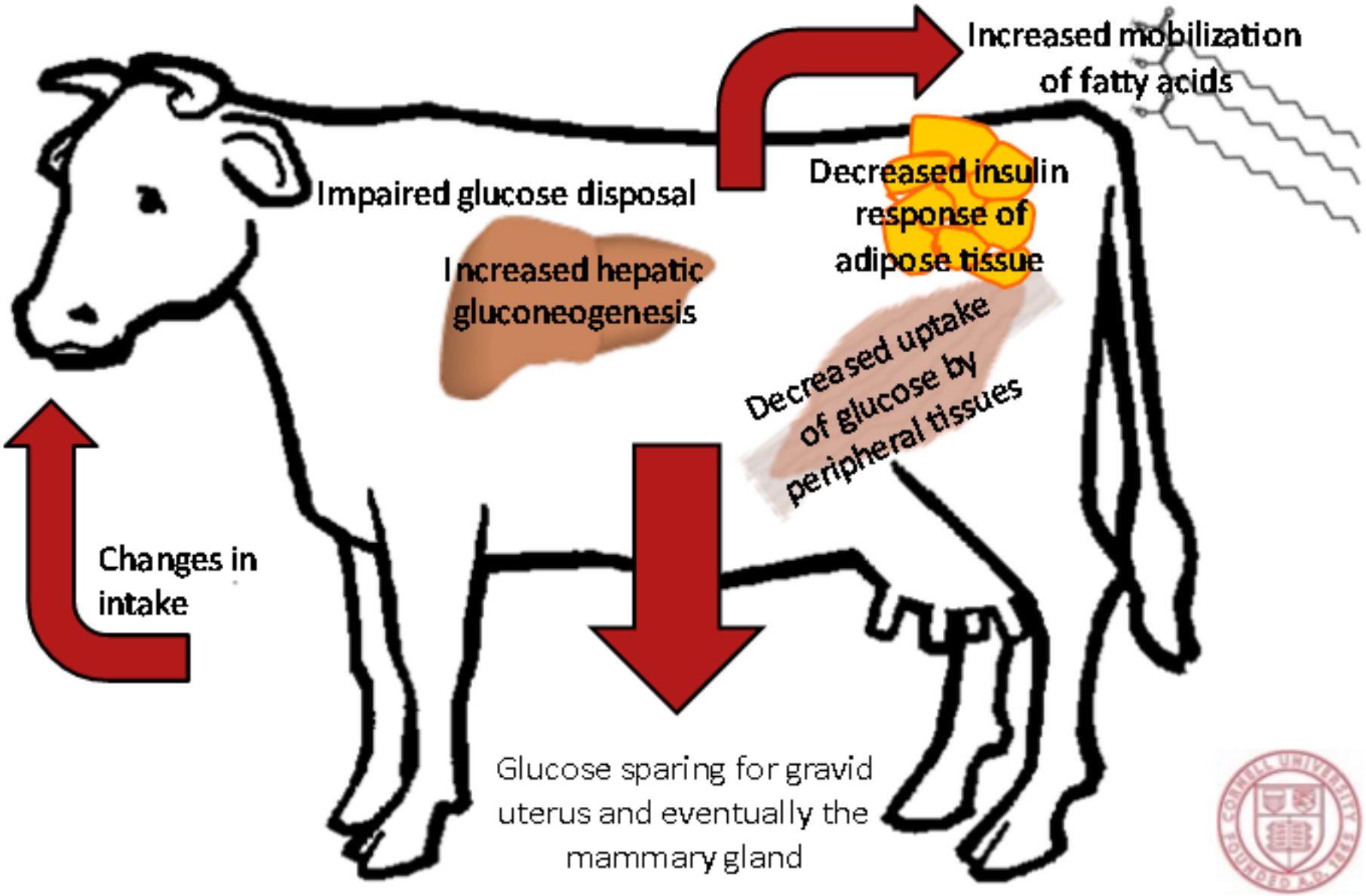
FATTY ACID SYNTHESIS VIDEO

- 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)



IS THIS NORMAL?

- 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

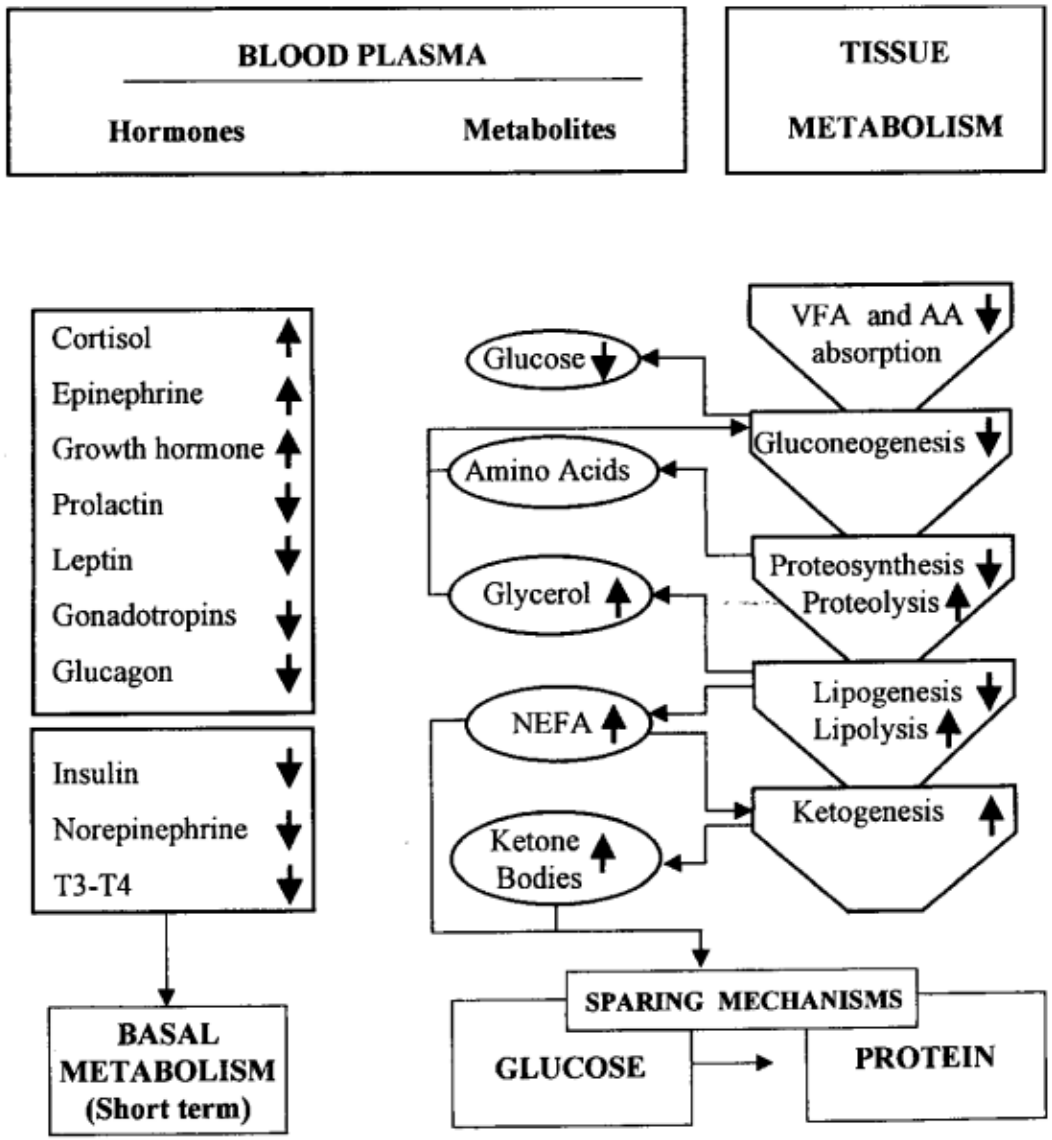


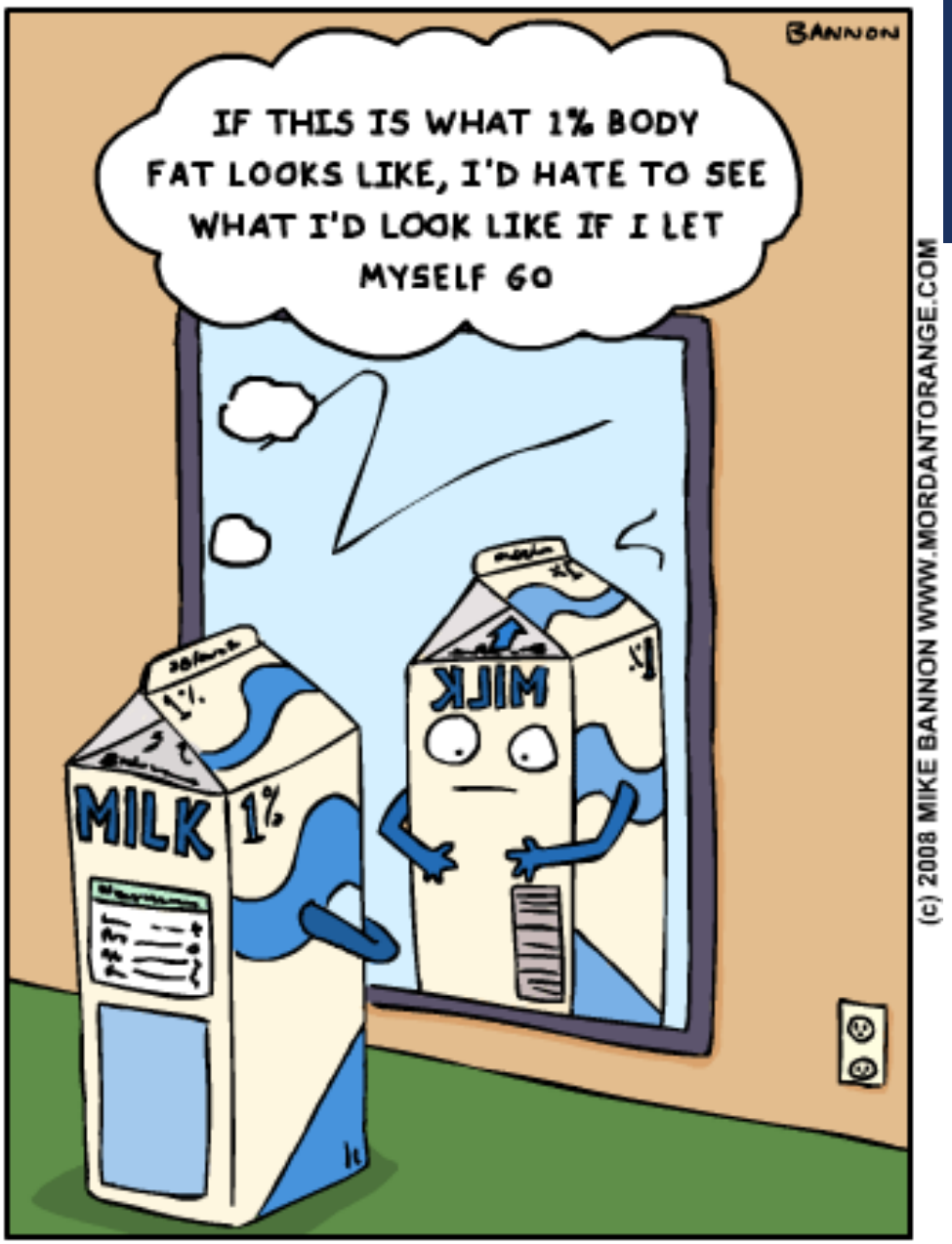
Figure 2. Metabolic and endocrine adaptations to undernutrition in the ruminant (AA, amino acids; NEFA, non-esterified fatty acids; VFA, volatile fatty acids; T3-T4, thyroid hormones).

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?


BANNON
IF THIS IS WHAT 1% BODY FAT LOOKS LIKE, I'D HATE TO SEE WHAT I'D LOOK LIKE IF I LET MYSELF GO
MILK 1%
MILK
(c) 2008 MIKE BANNON WWW.MORDANTORANGE.COM